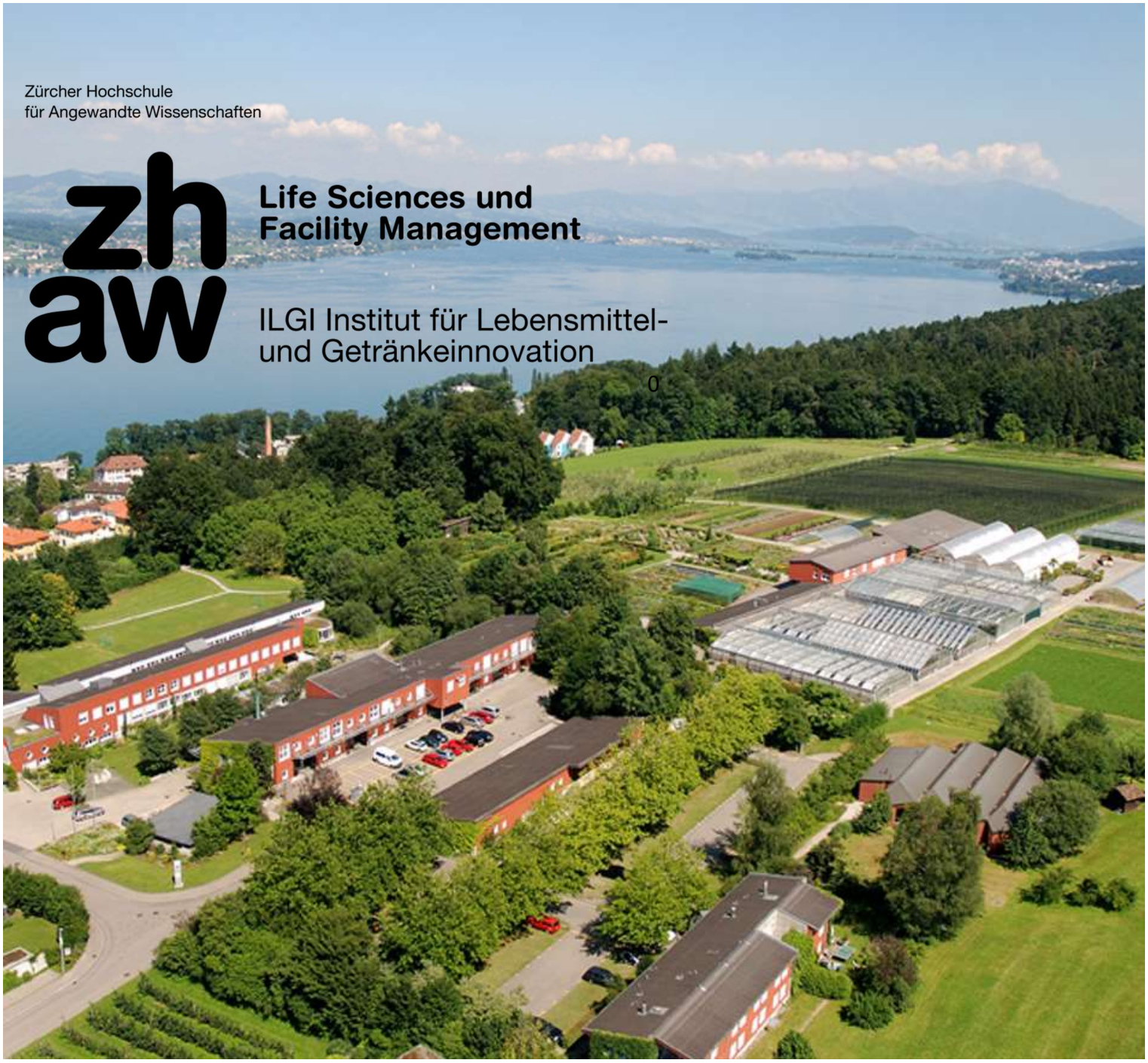


# Risk evaluation of *E. coli* ST 131 as a foodborne pathogen in Switzerland

Literature Review

by

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## Abstract

Within recent years, the topic of multidrug-resistant, uropathogenic *Escherichia coli* strains has seen a rise in occurrence as foodborne pathogens. At the core of this topic is the specific clonal group referred to as *Escherichia coli* O25b:H4 sequence type 131 (ST 131). It is an extended-spectrum β-lactamase (ESBL) producing *E. coli* strain, postulated to be responsible for the spread of ESBL-encoding genes world-wide. This literature review aimed to evaluate ST 131 as a foodborne pathogen in Switzerland, in order to assess the risk it poses for food producers in Switzerland specifically. On a global scale, ST 131 has been identified on all continents. It is mostly associated with chicken and poultry meat, and has been isolated from retail products many times. Not exclusive to chicken, it was rarely identified from fish guts and gills. In all other meat products, ST 131 was found only in faecal matter, not in the product itself. Besides meat, the pathogen was not identified from any sources such as dairy, fruit and vegetables. The situation is similar in Switzerland, where it was isolated frequently from chicken, rarely from fish and a complete absence of the pathogen in all other food product groups. The risk, ST 131 poses towards Swiss food producers has been evaluated as a medium risk factor for both chicken and fish products, and a low risk factor for any other products. Despite ST 131 being evaluated as a medium-to-low risk factor, depending on the product, it is still recommended to perform further research on the topic. Especially looking towards prevalence in Swiss food, in chicken and poultry meat, but also in fish. Specifically fish designated for raw consumption (Sushi, Sashimi). Moreover, the spread of aforementioned ESBL-encoding genes is hypothesized to occur also during infection of humans. This leads to the recommendation, that ST 131 should be considered a food-safety risk in all products, in order to eliminate said spread. Whether this consideration as a food-safety risk is feasible, cannot be said without further analysis of products and viable treatment options.

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## List of Abbreviations

| Abbreviation | Description |
|---|---|
| ESBL | Extended-Spectrum β-Lactamase |
| ST | Sequence Type |
| ExPEC | Extraintestinal Pathogenic |
| MLST | Multilocus sequence typing |
| EE broth | *Enterobacteriaceae* Enrichment Broth |
| PCR | Polymerase Chain Reaction |

# 1 Introduction and background

## 1.1 Introduction and goals of this review

Within recent times, the topic of multidrug-resistant *E. coli* strains, specifically sequence type 131 (ST 131) has seen a surge in occurrence as a foodborne pathogen. This has prompted the Swiss Federal Food Safety and Veterinary Office (FSVO) to investigate further into the problem. The Zurich University of Applied Sciences (ZHAW) was thus tasked with reviewing available literature, in order to answer and give insight to the following questions and topics.

a) Foodborne Origins – How did people get infected, which products were involved?
b) Situation in Switzerland – What is the state of occurrence in Switzerland specifically?
c) Risk evaluation – How big is the risk for the Swiss population?

In order to answer these questions, a thorough literature research is conducted, factoring in information from international as well as national sources. The following literature review first looks at the general problem of ST 131, while further narrowing information down to specific cases in Switzerland, and finally evaluating the risk it poses as a foodborne pathogen. The basis of information that started this review, stems from the ADURA ID Nr. 43 (FSVO, 2019).

## 1.2 *E. coli* sequence type 131 – general overview

*Escherichia coli* is a common yet diverse microorganism, living in the gastrointestinal tract of both human and animal. While commensal in its origin, *E. coli* has developed into a pathogen through both the loss and gain of genes (Croxen & Finlay, 2010). There are different pathogenic categories of *E. coli*, though they are mainly associated with intestinal infection. The six different categories of intestinal pathogenic *E. coli* are enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC) as well as diffusely adherent *E. coli* (DAEC). These pathogens are mainly associated with enteric or diarrhoeal disease. However, there are also pathogenic *E. coli* causing extraintestinal diseases. Urinary tract infections (UTI) are caused by uropathogenic *E. coli* (UPEC), while meningitis and sepsis are caused by meningitis-associated *E. coli* (MNEC) and sepsis-associated *E.coli* (SEPEC), re-

spectively. These three groups are collectively described as extraintestinal pathogenic *E. coli* (ExPEC) (Kaper et al., 2004). A visualization of the different categories of pathogenic *E.coli* is presented in Figure 1.

Extraintestinal pathogenic *E.coli* (ExPEC) are accompanied with a multitude of different hazards such as soft tissue or central nervous-system infection (Russo & Johnson, 2003). Moreover, they are now the leading cause for urinary tract infections, both community acquired and nosocomial (Nicolas-Chanoine et al., 2014). The burden on society in conjunction with ExPEC increases due to their developed resistances towards antibiotics. *E. coli* isolates, isolated within the last 25 years have seen a rise in resistance towards antibiotics such as fluoroquinolones and extended-spectrum cephalosporins. This, due to isolates producing extended-spectrum β-lactamases. It is suggested, that the spread of these resistances originates from a single clonal group, *E. coli* sequence type 131, an extraintestinal pathogenic, multi-drug-resistant *E. coli* (Coque et al., 2008; Hummers-Pradier et al., 2005; Johnson et al., 2010; Nicolas-Chanoine et al., 2014).

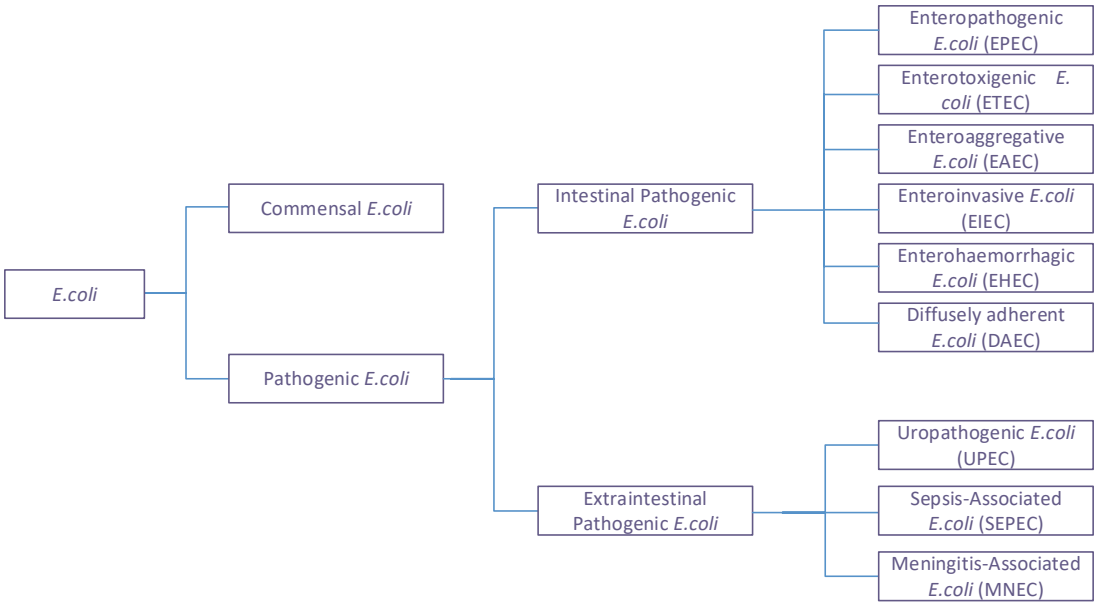


*Figure 1: Categorization of E.coli pathogens (Source: Wakeham, 2013, modified)*

## 1.3 Characterization & identification

Identification of ST 131 is carried out by multilocus sequence typing (MLST), with the defined seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA* and *recA*) or by screening for ST 131-associated single nucleotide polymorphisms (SNPs) in *mdh* and *gyrB* (Li et al., 2017). After initial characterization of *E. coli* O25:H4, ST 131, it was classified into phylogenetic group B2, containing strains both frequently

isolated from faeces of asymptomatic humans and from patients with urinary tract infections. This is important, as group B2 pathogens are known for harbouring comparatively more virulence factor-encoding genes than other groups. Virulence factors frequently characterized from ST 131 include but are not limited to *fimH* (type 1 fimbriae), *sat* (secreted autotransporter toxin), *fyuA* (yersinibactin receptor), *usp* (uropathogen-specific protein), *ompT* (outer membrane receptor), *iucD* (aerobactin), *iutA* (aerobactin receptor), *kpsM II* (group 2 capsule synthesis), *iha* (adhesin siderphore receptor), *tratT* (serum resistance associated) and *malX* (pathogenicity island marker). Out of these virulence factors, *kpsM II* and *iutA* are further two of the global five molecular factors used in defining ExPEC (Nicolas-Chanoine et al., 2014). Besides ST 131, a newly emerging clonal group, ST 1193, has been isolated in Europe, North America and Asia. Specifically in Germany, this clonal group was first identified in 2019, and was characterized as already resistant towards third-generation cephalosporins (Valenza et al., 2019).

## 1.4 Multidrug-resistance

As previously stated, the reoccurring problem associated with ST 131 is its developed multidrug resistance. There are two main resistances commonly found ST 131. The first characteristic is resistance toward extended-spectrum cephalosporins, brought upon by the production of extended-spectrum β-lactamase CTX-M-15 (Coque et al., 2008; Nicolas-Chanoine et al., 2008). The resistance mechanisms in ST 131 extends to fluoroquinolones and aminoglycosides (Rogers et al., 2011).

## 1.5 Pathogenic characteristics & pathogenesis

ST 131 are reported to cause different diseases. Generally, Urinary tract infections and bacteraemia are most commonly associated with it. Further, intra-abdominal and soft tissue infections have been reported. Meningitis, osteoarticular infections, septic shocks and epidymo-orchitis have also been seen as a result of ST 131 infections (Nicolas-Chanoine et al., 2014). Looking at its pathogenesis, ST 131 isolates are reported to have multiple factors for virulence potential. Biofilm production was studied to be more prevalent in ST 131 samples compared to non-ST 131 (Clermont et al., 2008). They are further reported to have increased metabolic potential. This in turn increases their potential to establish and maintain intestinal colonization. Despite overwhelming evidence of pathogenicity of ST 131, no correlation between pathogenic properties and virulence factor-gene carrying has been found (Nicolas-Chanoine et al., 2014). Rates of hospitalization and mortality are generally linked to different symptoms and complications in case of infections. Mortality rates in case

of bacteraemia, onset via ESBL producing organisms such as ST 131 range from 20% to 40%, depending on the case and time frame (Chung et al., 2012; Tumbarello et al., 2007). Hospitalization rates for ST 131 patients are inconclusive and no substantial data was found, since in all cases, an infection with ST 131 is only confirmed upon the subject being hospitalized or in a respective follow-up study, as there is no difference in the clinical picture between an UTI caused by ST 131 versus non-ST 131 infectious UTI (Doi et al., 2013; Mills et al., 2020). For mortality rates of ST 131 infections, some data can be found. A study by John P. Mills et al. compared different infections of ESBL producing *E. coli* from Detroit, USA. A total of 369 infections were accounted in the study, with 96% of infections determined as ST 131 infections. Across both ST 131 and non-ST 131 infections, a uniform 90-day mortality rate of 8% was found. While this is a rather staggering number, it is noteworthy, that patients included in this study had a mean age of 68, and more than half of the patients resided in either nursing homes or were hospitalized shortly before conducting the study (Mills et al., 2020). A different study, analysed patients involving bacteraemia cases associated with ESBL producing organisms, including 36 cases of ST 131 and 86 cases of non-ST 131. Mortality rates were staggeringly higher in this case, ranging from 22.2 % (n = 8) 14-day mortality in ST 131 infections to 25.0 % (n = 9) 28–day mortality rate. While high, these mortality rates did not differ greatly from non-ST 131 infections (22.1 % and 24.4 % respectively). It is further noteworthy, that this study only included cases of ST 131 infections causing bacteraemia, and most patients had other pre-existing conditions (Chung et al., 2012). Mortality rates of patients infected with ST 131, are further associated with a range of comorbidity issues such as hypertension, diabetes mellitus, malignancy and other. A study conducted in 2018 compared the outcome of 814 patients suffering from community-onset monomicrobial *E. coli* bacteraemia. Out of the 814 patients, 102 (12.1 %) were determined to be infected by ST 131. Mortality rates in this study were evaluated for ST 131 as 10.8 % (n = 11) for 14-day period and 14.7 % (n = 15) for 28-day period, compared to non-ST 131 at 4.3 % (n = 32) and 6.5 % (n = 48) respectively. This study also concluded, that ST 131 poses a greater risk for the elderly, as well as patients with pre-existing conditions (Wang et al., 2018). Conclusively, mortality rates for ST 131 infections, found in literature range from 8 % to 25 %. However, there is little to no information available for patients with no comorbidity factors, thus inflation of mortality rates is a possibility.

# 2 Methods & Results

The following chapter first presents methodology during literature research and further aims to summarize results from available literature. While first, the pathogen will be tracked on a global scale, it is further narrowed down to cases specifically recorded in Switzerland.

## 2.1 Methodology

Literature research was performed during a time frame, starting February 5th until May 29th. Articles on the specific topics were found through database research, mainly using Web of Science, Science Direct and Google Scholar. Keywords were all used on different databases and in combination with each other. Filtering of results was performed via relevance or sort by newest setting, according to the specific database filtering algorithm. Choice of filtering algorithm was based on the topic of individual search entries, and was performed in parallel for most key words combinations, in order to account for both new and older research entries. A list of used keyword combinations and hits on different databases can be found in Table 1. It is noteworthy, that this list only includes search term combinations used for assessing the risk ST 131 poses, not for any background information as seen in Chapter 1, and that the list is not complete to the full extent of this review.

*Table 1: Keyword combinations & database hits*

| **Keywords** | | | **Pub-Med** | **Google Scholar** | **Web of Science** |
|---|---|---|---|---|---|
| *E. coli* | ST131 | Food | 85 | 3’500 | 73 |
| *E. coli* | ST131 | Clinical | 384 | 6’470 | 329 |
| *E. coli* | ST131 | Animal | 82 | 3’590 | 56 |
| *E. coli* | ST131 | Chicken | 26 | 1’640 | 13 |
| *E. coli* | ST131 | Meat | 33 | 1’670 | 50 |
| *E. coli* | ST131 | Beef | 3 | 543 | 2 |
| *E. coli* | ST131 | Pork | 3 | 435 | 3 |
| *E. coli* | ST131 | Dairy | 1 | 687 | 2 |
| *E. coli* | ST131 | Milk | 1 | 696 | 2 |
| *E. coli* | ST131 | Fruit | 3 | 228 | 3 |

| Keywords | | | Pub-Med | Google Scholar | Web of Science |
|---|---|---|---|---|---|
| *E. coli* | ST131 | Vegetable | 3 | 165 | 3 |
| *E. coli* | ST131 | Retail | 17 | 1’390 | 24 |
| *E. coli* | ST131 | Foodborne | 14 | 1’370 | 5 |
| *E. coli* | ST131 | Outbreak | 29 | 2’650 | 52 |
| *E. coli* | ST131 | Poultry | 38 | 1’800 | 41 |
| *E. coli* | ST131 | Fish | 5 | 616 | 4 |
| *E. coli* | ST131 | Companion Animal | 8 | 209 | 7 |
| *E. coli* | ST131 | Occurrence | 55 | 3’310 | 53 |
| *E. coli* | ST131 | Swiss | 4 | 412 | 1 |
| ST131 | Swiss | Food | 3 | 308 | 1 |
| ST131 | Swiss | Clinical | 1 | 421 | 0 |
| ST131 | Swiss | Animal | 1 | 302 | 0 |
| ST131 | Swiss | Chicken | 0 | 174 | 0 |
| ST131 | Swiss | Meat | 0 | 205 | 0 |
| ST131 | Swiss | Beef | 0 | 67 | 0 |
| ST131 | Swiss | Pork | 0 | 54 | 0 |
| ST131 | Swiss | Dairy | 0 | 96 | 0 |
| ST131 | Swiss | Milk | 0 | 115 | 0 |
| ST131 | Swiss | Fruit | 1 | 31 | 1 |
| ST131 | Swiss | Vegetable | 0 | 28 | 0 |
| ST131 | Swiss | Retail | 0 | 162 | 0 |
| ST131 | Swiss | Fish | 0 | 89 | 0 |
| ST131 | Swiss | Poultry | 0 | 189 | 0 |
| *E. coli* | ESBL | Animal | 416 | 16’500 | 229 |
| *E. coli* | ESBL | Chicken | 150 | 5’370 | 234 |
| *E. coli* | ESBL | Fish | 27 | 3’320 | 28 |
| *E. coli* | ESBL | Poultry | 208 | 6’320 | 231 |
| *E. coli* | ESBL | Food | 438 | 17’300 | 414 |

## 2.2 Clinical studies & Companion animals – Worldwide

Tracking ST 131 in order to create a timeline of its first identification and its development until today is a vague task. The first instance of a foodborne pathogen causing urinary tract infection can be traced back to 1970 (Shooter et al., 1970), though the idea was not further developed. In 1994 and later the following decade however, a number of outbreaks were recorded, linking UTI to strains of multidrug-resistant *E. coli* (Manges et al., 2001, 2007; Olesen et al., 1994). Later, two independent research groups identified *E. coli* serogroup O25b ST 131 on three different continents as a main factor in spreading ESBL encoding genes (Coque et al., 2008; Nicolas-Chanoine et al., 2008).

Clinical studies regarding UTI infections with ST 131 are widely available and can now be traced on all continents across many countries, with new studies published as late as of January 2020 (Pitout & Finn, 2020; Rogers et al., 2011).

When looking at clinical studies or outbreaks in general, the source of origin differs from case to case. Ways of transmission include, but are not limited to dissemination through environment (Sabaté et al., 2008), carried by domestic or wild animals (Ewers et al., 2007; Guenther et al., 2010), person to person transmission (Ender et al., 2009) and faecal carriage, linked to foodborne origins (Platell, Johnson, et al., 2011; Vincent et al., 2010).

On the topic of domestic and companion animals, ST 131 has been isolated frequently. Studies conducted analysed isolates ranging from cats, dogs, to guinea pigs birds, and horses. Results show ST 131 being isolated from companion animals on a global scale, and from different animals. The number of cases infected with ST 131 are infrequent however. While ST 131 has been found in companion animals, they are not considered a primary way of dissemination and spread of ESBL producing isolates or ST 131 (Nicolas-Chanoine et al., 2014; Platell, Cobbold, et al., 2011; Platell, Johnson, et al., 2011).

In conjunction with domestic animals, a 2015 study from Germany focused on a potential link between livestock animals and farm workers infected by ESBL producing *E. coli*. A total of 34 different farms were tested, including farms raising pigs (n= 17, 50 %), cattle (n = 11, 32.4 %), chicken (n = 4, 11.7 %) and turkey (n = 2, 5.9 %). Further, a total of 73 farm workers participated in the study. 70.6 % of all farms (n = 24) showed livestock harbouring ESBL producing *E. coli*. Positive ESBL producing organisms were found in pig farms (n = 6, 54.5 %), cattle farms (n = 15, 88.2 %) and chicken farms (n = 3, 75 %). No positive samples were found in turkey farms. On the side of the farm workers, only people working with cattle (n = 3, 12.5 %) and

pigs (n = 2, 6.3 %) were affected by ESBL producing *E. coli*. The study thus concluded, that epidemiological links between livestock and farm workers are likely to exist, and that livestock animals may very well be a reservoir of ESBL-organisms (Dahms et al., 2015).

## 2.3 Occurrence – Foodborne origins worldwide

The foodborne origins of ST 131 infections on an international scale can be traced back to a multitude of different sources, though most are animal-based. Identification of ST 131 as a foodborne pathogen can be traced globally. Examples for worldwide isolation of ST 131 from food can be seen below.

A study published in 2010 analysed *E. coli* isolates from retail foods, restaurant products as well as clinical samples in Canada, from the Quebec and Ontario region. Foodborne isolates included chicken, beef, pork, fish, seafood and non-animal products such as pasta, vegetables and fruit. A total of 844 *E. coli* isolates were analysed, with foodborne isolates accounting for 58% of all samples. Only one isolate from chicken and two clinical samples indicated the presence of ST 131, though other clonal groups harbouring ESBL genes were found as well (Vincent et al., 2010). Due to the nature of this study, only analysing isolates and not products themselves, no clear conclusion can be drawn on the contamination level of the retail products.

Similar, a 2013 study analysed *E. coli* isolates from retail chicken breast in the USA. A total of 175 strains were analysed first for molecular criteria of ExPEC. 25 were determined as ExPEC (14.3% of total strains). Further, ExPEC strains were analysed for phylogenetic groups or clonal group. 5 strains (20% of ExPEC strains, 2.8% of all strains) came out as ST 131 (Johnson et al., 2017).

A 2015 study from Italy analysed chicken broiler meat for ESBL producing isolates. A total of 163 samples, originating from retail stores in Palermo, Italy were analysed. Of all samples, 109 (66.9%) harboured isolates resistant towards certain antibiotics. In total, 134 *E. coli* strains were isolated which were tested positive for ESBL production. Though ESBL producing *E. coli* were thus frequent, only 4 (3%) isolates were confirmed as ST 131 (Ghodousi et al., 2015).

Occurrence of ESBL producing *E. coli* was also investigated in a 2018 study in Thailand. A total of 250 samples were collected in open air (n = 103) and supermarkets (n = 147) in the Phitsanulok province. Samples consisted of chicken (n = 218), duck (n=14) and other birds (n = 18). Samples were analysed for ESBL producing *E. coli* strains. A total of 100 samples (40%) harboured ESBL producing *E. coli*. Strains

were analysed further for the phylogenetic group. While no specific detection was for ST 131 was performed, only 4 samples (4%) belonged to phylogenetic group B2, which ST 131 is a part of (Tansawai et al., 2018).

All examples stated above show clear indication of ESBL producing *E. coli* in food being mainly associated with chicken meat. Studies regarding other foods have been conducted as well. A study from Denmark, analysing *E. coli* samples from UTI patients, chicken, pork and pigs concluded that ESBL producing *E. coli* are also harboured in pigs or production animals, though not as prevalent as in chicken or poultry (Jakobsen et al., 2010).

Besides pork, fish from the Mediterranean Sea were analysed in a 2015 study from France, analysing a total of 300 samples of fish guts and gills. 22 Samples (7.3%) harboured ESBL producing *E. coli*. Of all ESBL producing samples, 3 (13.6 %) belonged to phylogenetic group B2, of which 2 isolates were described as ST 131 (Brahmi et al., 2015).

Besides chicken, pork and fish, no information was found about ST 131 being isolated from other foods such as beef, dairy or non-animal based products. Table 2 summarizes all results mentioned in this chapter.

*Table 2: Summary of international ST 131 findings.*

| Sample type | Origin | Amount of samples tested | ESBL isolates | ST 131 isolates | Source |
|---|---|---|---|---|---|
| *E. coli* isolates from different sources | Canada | 844 (490 food isolates) | N/A | 3 (1 from chicken meat, 2 clinical isolates) | Vincent et al., 2010 |
| *E. coli* isolates from chicken breast | USA | 175 | N/A | 5 | Johnson et al., 2017 |
| Chicken broiler meat | Italy | 163 | 134 | 4 | Ghodousi et al., 2015 |
| Chicken, duck, other birds | Thailand | 250 | 100 | N/A | Tansawai et al., 2018 |
| Fish guts and gills | Mediterranean Sea | 300 | 22 | 2 | Brahmi et al., 2015 |

## 2.4 Occurrence – Switzerland

As of writing this review, a general wide-spread outbreak of ST 131 has not been recorded. Generally, literature available for cases of the pathogen in Switzerland is scarce. There have been, however, a few instances worth mentioning.

As previously stated, chicken seems to be the focus when looking at ST 131 as a foodborne pathogen. A 2014 study analysed Swiss and imported poultry meat. A total of 9 samples were bought in Swiss supermarkets, containing 4 samples of Swiss chicken and 5 samples of imported meat (Hungary and France). Despite the small number of samples, a total of 24 extended-spectrum cephalosporin-resistant *E. coli* strains were isolated from the 9 samples. Of these 24 samples, 11 (46%) were further characterized as ESBL producing. After further characterization, none (n = 0) were described as ST 131 (H. Abgottspon et al., 2014).

A more broad scale study was conducted in Switzerland, analysing ESBL producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. Samples consisted of cattle (faecal samples, n = 124), pigs (faecal samples, n = 59), chicken (faecal samples, n = 93), minced meat (pork and beef, n =104), bulk tank milk (n = 100), as well as *E. coli* isolates from mastitis milk (n = 67). Samples of bulk tank milk and minced meat were all tested negative for ESBL producing *Enterobacteriaceae*. There were however ESBL producing isolates characterized from all other sources, as seen in Table 3.

*Table 3: ESBL producing isolates from different food and animal sources. Data source: (Geser et al., 2012)*

| **Origin** | **Number of samples tested** | **Number of ESBL producers (percentage)** |
|---|---|---|
| Cattle, faecal samples | 124 | 17 (13.7 %) |
| Pig, faecal samples | 59 | 9 (15.3 %) |
| Chicken, faecal samples | 93 | 59 (63.4 %) |
| Sheep, faecal samples | 58 | 5 (8.6 %) |
| Minced meat | 104 | 0 (0 %) |
| Bulk tank milk | 100 | 0 (0 %) |
| Isolates from mastitis milk | 67 | 1 (1.5 %) |

ESBL producing isolates were further analysed for specific resistance genes, though no multilocus sequence typing was performed. Whether ST 131 was among found ESBL producing isolates is inconclusive (Geser et al., 2012).

In 2014, a study was published, describing the isolation of ST 131 from fish. Samples were obtained from two lakes in Switzerland, Lake Zurich and Lake Thun. A total of 139 samples were analysed, consisting of *Coregonus lavertus* (whitefish, 64 samples), *Perca fluviatilis* (perch, 33 samples), *Rutilus rutilus* (roach, 29 samples), *Salmo trutta* (brown trout, 6 samples), *Esox Lucius* (pike, 4 samples), *Abramis brama* (bream, 1 sample), *Tinca tinca* (trench, 1 sample) and *Centrarchidae* (sunfish, 1 sample). Samples were analysed using the guts. Of all samples, a total of 26 samples (18.7%) showed 33 different isolates producing ESBL or pAmpC. Most isolates were *E. coli*. A total of 7 isolates were identified as *E. coli* ST 131 (Helga Abgottspon et al., 2014).

In conjunction with the analysis of fish, in 2013, a study was conducted sampling water from 58 different lakes and rivers in Switzerland. Samples were taken from the German speaking part of Switzerland and screened for ESBL- and carbapenemase- producing *Enterobacteriaceae.* Of all locations, 21 (36.2%) showed ESBL producing isolates and 1 showed carbapenemase producing isolates. The total of 74 isolates producing ESBL were mainly *E. coli* (n = 60).(Zurfluh et al., 2013).A follow-up study later analysed obtained ESBL isolates further. Three strains of ST 131, each harbouring different ESBL encoding genes were characterized. However, no information is available, concerning how many samples were tested positive for these exact strains (Müller et al., 2016).

Despite scarce information concerning the occurrence of ST 131 in non-animal based food on international scale, a study was conducted in Switzerland, targeting ESBL producing *Enterobacteriaceae* in Ready-to-eat (RTE) salads, fresh-cut fruit and sprouts. A total of 238 mixed and unmixed RTE salads and 23 sprout samples were analysed as well as 4 samples of recycled wash water from production plants. Out of the 238 salad products, 12 (5%) harboured ESBL producing *Enterobacteriaceae*. Though none were *E. coli* isolates. One samples of recycled wash water from the production plant yielded ESBL producing isolates. This isolate was further characterized to be of phylogenetic group B2 and in fact ST 131 (Nüesch-Inderbinen et al., 2015).

Further on the topic of vegetables, a 2015 studies analysed ESBL isolates from imported vegetables from India, Thailand, Vietnam and the Dominican Republic. Analysed were a range of different products, including cucumbers, leafy greens and eggplant. A total of 169 samples were analysed for ESBL producing organisms. 43 samples (25.4 %) harboured one or more ESBL producing *Enterobacteriaceae*, while 78.3 % of these isolates were multidrug-resistant. Out of all isolates, one single *E .coli* strain was further characterized as ST 131, stemming from a bitter cucumber from the Dominican Republic (Zurfluh et al., 2015).

Summarising all results from studies conducted in Switzerland, a similar picture as international levels can be drawn. Products mainly associated with ST 131 are chicken and poultry meat, followed by fish. Beef, pork and cattle as well dairy and non-animal based products see small to no focus of identification. Table 4 summarizes all results from Swiss studies.

*Table 4: Summary of ST 131 findings in Switzerland.*

| Sample type | Origin | Amount of samples tested | ESBL isolates | ST 131 isolates | Source |
|---|---|---|---|---|---|
| Chicken | Switzerland<br>France<br>Hungary | 9 | 24 | 0 | Abgottspon et al., 2014 |
| Freshwater fish | Switzerland | 26 | 33 | 7 | Abgottspon et al., 2014 |
| Freshwater from lakes and rivers | Switzerland | 58 | 74 | 3 | Zurfluh et al., 2013, Müller et al., 2016 |
| RTE salads and sprouts | Switzerland | 261 | 12 | 0 | Nüesch-Inderbinen et al., 2015 |
| Recycled washing water | Switzerland | 4 | 1 | 1 | Nüesch-Inderbinen et al., 2015 |
| Cattle, faecal samples | Switzerland | 124 | 17 | N/A | Geser et al., 2012 |
| Pig, faecal samples | Switzerland | 59 | 9 | N/A | Geser et al., 2012 |
| Chicken, faecal samples | Switzerland | 93 | 59 | N/A | Geser et al., 2012 |
| Sheep, faecal samples | Switzerland | 58 | 5 | N/A | Geser et al., 2012 |
| Minced meat | Switzerland | 104 | 0 | N/A | Geser et al., 2012 |
| Bulk tank milk | Switzerland | 100 | 0 | N/A | Geser et al., 2012 |
| Isolates from mastitis milk | Switzerland | 67 | 1 | N/A | Geser et al., 2012 |
| Vegetables (multiple) | Thailand, Vietnam, India, Dominican Republic | 169 | 43 | 1 | Zurfluh et al., 2015 |

# 3 Discussion and outlook

## 3.1 Risk evaluation – general overview

The focus of this study was to obtain information on ST 131 as a foodborne pathogen both on international level and specifically in Switzerland. While information is available, the focus of most studies is not on outbreaks of the pathogen. Current research is more aimed towards resistance mechanics and spread of ESBL encoding genes. While the spread of ST 131 through food and companion animals has been recognized (Platell, Johnson, et al., 2011), it is not of utmost importance, as a wide spread outbreak has not been recorded as of writing this review, both in Switzerland and internationally. Further, ST 131 is only part of the emerging ESBL producing *Enterobacteriaceae*-problem that is emerging.

Products that are affected by ST 131 are mainly chicken and poultry meat, as well as fish in rare cases. While production animals such as pigs, sheep and cattle also seem to harbour ESBL producing organisms, retail products were rarely affected in studies mentioned in previous chapters and isolates were mainly obtained from faecal samples. This however sets a dangerous position for food producers and the consumer alike.

## 3.2 Risk evaluation – ST 131 as a foodborne pathogen

Risk evaluation of ST 131 as a foodborne pathogen is evaluated by the assistance of a risk matrix. The risk matrix evaluates the threat of ST 131 based on the factors probability of infection and severity of outcome. In all cases, the severity of an infection of ST 131 and thus the severity of outcome, can be evaluated at a medium risk. This due to the fact, that infections with ST 131 are rarely fatal, however complications may still arise due to the aforementioned antibiotics resistance, as previously mentioned. The probability of an infection is further evaluated via literature found in chapter 2, and is estimated per product or product group. Combining probability and severity, the risk matrix evaluates a rating, ranging from low risk factor to high risk factor, which indicates general safety of a product to the consumer on the topic of ST 131.

### 3.2.1 Risk evaluation – Chicken & poultry

Chicken and poultry is the product most frequent associated with harbouring ST 131. Looking at literature, analysing chicken for ST 131, it is the product where occurrence is observed most frequent. Similar to the previous category, contamination of chicken with pathogens occurs during the slaughtering process. In contrast, contamination can in some cases not be avoided, thus chicken being generally marketed as a product that requires a thorough heating process. Looking at available retail products, it is important to distinguish between two different categories, convenience food and raw chicken products. Convenience products such as chicken nuggets or similar that already endured a heating process are in no risk for ST 131 contamination, as manufacturers of these products are already tasked with combating contamination of *Salmonella* or *Campylobacter.* Appearance of ST 131 should thus not interfere with the production process, as it can be inactivated in parallel to the aforementioned pathogens. On the other hand, ST 131 poses a greater threat to consumers purchasing fresh or raw chicken products. The problem therein lies in the processing by the customer at home, where regulations are difficult if not impossible to influence. ST 131 being an ExPEC, the problem lies less in insufficient heating of the product, but more in cross-contamination risk via utensils. Manufacturers of these products are thus at higher risk of providing contaminated product that may cause eventual infection and thus a greater risk for the consumer. This puts the probability of infection at a high level and thus for the case of raw chicken, ST 131 is evaluated as a medium risk factor. As in combination with a medium severity of outcome, it is still not considered high risk factor (Figure 2).

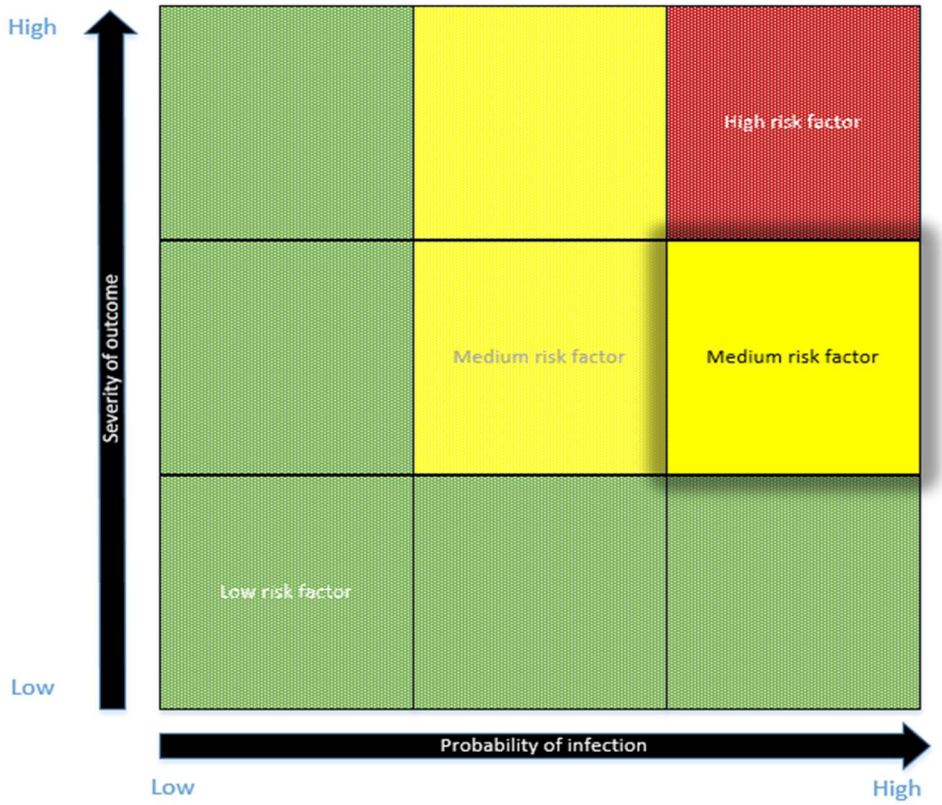


*Figure 2: Risk evaluation matrix category chicken & poultry.*

### 3.2.2 Risk evaluation – Fish, Seafood

Fish and Seafood has been analysed infrequently for ST 131. In the two cases, where ST 131 was found, only fish guts and gills were analysed, not product itself. The single study analysing fish and seafood isolates found no ST 131 among *E. coli* isolates. Risk evaluation for Fish and seafood has to be differentiated between product consumed after a heating process and product consumed raw. Products consumed after a thorough heating process pose a medium risk for consumers. Compared to chicken and pork, fish has a tendency to be consumed before reaching critical heating temperature of 72°C. Further, cross contamination of raw product with utensils may be a potential way of dissemination. Products designated for raw consumption, such as Sushi or Sashimi pose a much greater risk if ST 131 were to be isolated frequently, as these products, as suggested, are not heat treated. A similar case is that of seafood. Depending on the product, it may be consumed raw (e.g. oysters) or after heat treatment. Conclusively, there is currently not enough information available in order to make a clear statement for risk assessment for consumers in Switzerland. Information necessary in order to evaluate products

should include contamination numbers from Swiss fish, but as well from international sources. Especially due to freshwater and thus Swiss fish being rarely used for Sushi, Sashimi or similar Asian-inspired, raw consumed products. Should the case arise of high contamination levels in fish or seafood, ST 131 might be considered a food safety risk for products designated for raw consumption and a process-hygiene criteria for products that are to be heat treated during processing. For the time being, fish and seafood can be considered a medium risk factor, since the probability of infection is considered medium at most, until further information is obtained (Figure 4).

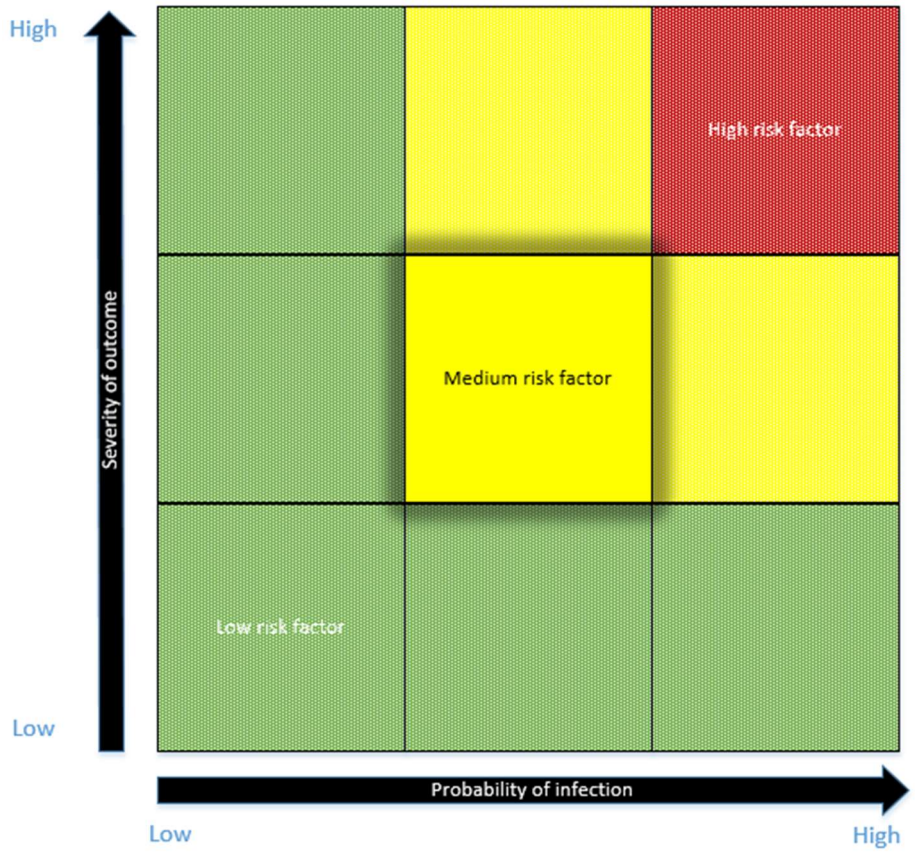


*Figure 3: Risk evaluation matrix category Fish & Seafood.*

### 3.2.3 Risk evaluation – Beef, veal & pork

The risk evaluation for beef, veal & pork are evaluated in the same risk matrix (Figure 2). Contamination of these products with more common foodborne pathogens such as STEC happens usually during the slaughtering process. This is not different from the ST 131 case. Despite findings of ST 131 in faecal samples of cattle and pigs, no products contaminated with the pathogen were found. Though from the contaminated faecal matter to contaminated product might not be a far leap, as long

as GMP regulations are upheld, the risk for Swiss consumers, purchasing beef, veal and pork on the topic of ST 131 can be considered low, since the absence of pathogens in the products implicates a low probability of infection. This evaluation is based on current findings of ST 131, and may change, based on whether ST 131 can be isolated more frequently from Swiss products specifically. In the case of high isolation numbers, the risk would be considerably higher, since especially beef & veal is frequently consumed without reaching a critical core temperature of 72°C, and in some cases even raw. Would ST 131 be isolated frequently from beef or veal, it might be considered a food safety risk.

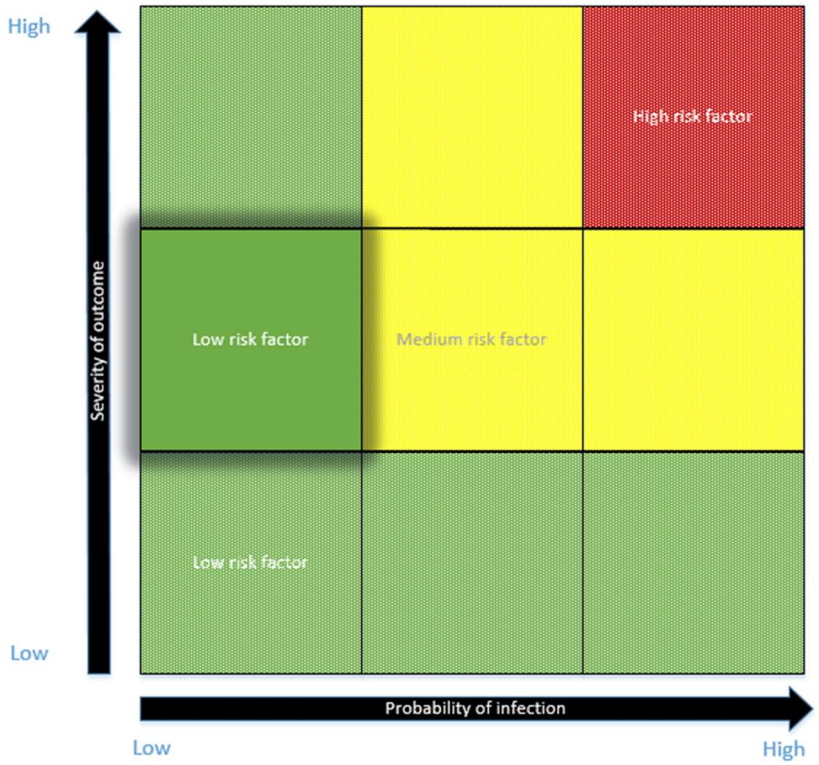


*Figure 4: Risk evaluation matrix category beef, veal & pork...*

### 3.2.4 Risk evaluation – Remaining food products

The last category, titled as “remaining food products” includes everything from dairy, fruit and vegetables to read-to-eat products. This wide spread of different products being evaluated in the same manner is justified due to the scarcity of available literature and in combination with the lack of finding of ST 131 in the few cases that are available. All these products can be evaluated as low risk factors as of now, simply because the few studies that analysed products other than fish or chicken, did not find any positive samples. Further, ST 131 being found most frequently in faecal

samples of animals, contamination is improbable, as long as GMP standards are upheld during the processing of animal-based products. For non-animal based products, such as fruit and vegetables, the risk for ST 131 contamination is deemed highly unlikely (Figure 5). Finally, during extensive testing of wheat flour and whole wheat grain during a project involving analysis for Shiga Toxin-Producing *Escherichia coli*, in a project performed at the Federal Food Safety and Veterinary Office, no sample was tested positive for ST 131, further supporting the claim of a low risk factor for wheat based products in particular. (R. Boss, FSVO, personal communication, June 16, 2020).

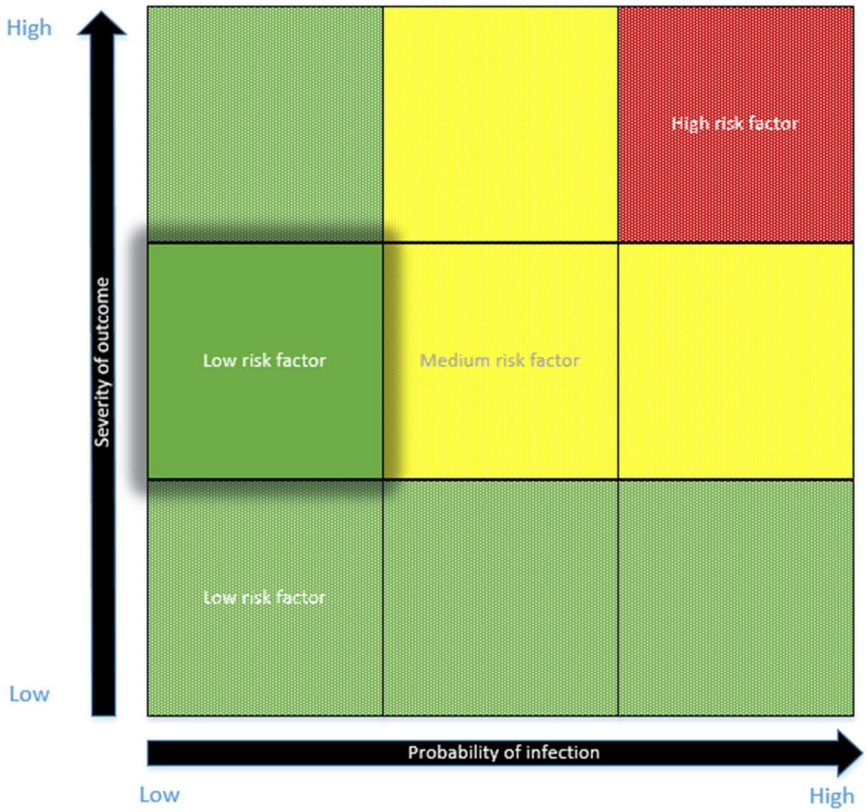


*Figure 5: Risk evaluation matrix category "remaining food".*

### 3.2.5 Risk evaluation – Conclusion

In conclusion, Chicken as well as fish and seafood can be considered products that pose an elevated risk of being the source for a potential ST 131 infection. In order to get a better overview of the spread, it is thus recommended to start with these two product categories, in case of a country-wide campaign that aims to assess the risk of ST 131 on a numerical level. After this threat has been evaluated, beef and pork should be next to be taken a closer look at. Since faecal samples have seen positive results for ST 131, the risk still exists, while low, as no retail products have

been analysed as positive. As of writing this review, it is not recommended to further analyse products other than the aforementioned, especially non-animal based products such as fruit and vegetables.

## 3.3 Outlook

The aim of this literature review was to determine how big of a risk ST 131 poses as a foodborne pathogen. Specifically in Switzerland it is apparent, that ESBL producing organisms, as well as ST 131 have been isolated from products, thus the organism posing a potential threat. For consumers of chicken and poultry, but further fish and seafood, ST 131 poses a threat. Despite information about rates of contamination for products being scarce, producers should aim to eliminate or minimalize the risk for potential infections in customers. The problem hereby arises for products that are sold in a raw state. Similar to other *Enterobacteriaceae,* complete absence of the bacteria without a heat treatment is difficult. Looking at the pathogenicity of ST 131 versus more well-known contaminants, a few statements can be made. Mortality rates of infections with *E. coli* are generally low. However, looking at ExPEC in conjunction with clinical cases, mortality rates spike when complications such as sepsis arise (Russo & Johnson, 2003). Combining this with the ever increasing number of antibiotics resistances, ST 131 specifically, the threat it poses will increase as time goes on. It is postulated, that a main way of spreading ESBL encoding genes occurs in infected patients, as ST 131 has adapted to the human host (Nicolas-Chanoine et al., 2014). Understanding the threat ST 131 poses, not only as a pathogen, but as a vehicle for spreading ESBL encoding genes, the authors have come to a conclusion. The aim should be, to eliminate ST 131 from commercial products as far as possible. This stems from not only it's pathogenic threat, but also from its ability to spread ESBL encoding genes, which has a high likelihood of developing into a problem, that reaches far beyond the food sector. Eliminating this vector of resistance spreading in the food supply chain, may be a key factor in deterring a global, emerging problem. It is thus recommended, to consider ST 131 a food-safety risk for both Swiss and imported products, and independent on the designated usage (raw consumption versus consumption after heat treatment). Further, for chicken, it is difficult to say what the appropriate action should be. Chicken being most frequently associated with ST 131 contamination, it is difficult to say, whether complete absence of the bacterium is feasible, similar to *Campylobacter jejuni.* This would have to be confirmed in follow-up studies looking directly at contamination rates. Considering ST 131 a food-safety issue in chicken, not only due

to pathogenicity, but also due to the spread of ESBL encoding genes is still recommended by the authors.

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# 5 Appendix

## 5.1 Identification – Recommendations for laboratories

Should the recipient of this review decide to further investigate or task laboratories cross-country with evaluating food products, the following chapter aims to help this endeavour. Since a country-wide campaign would ask for multiple laboratories to collaborate, a unified evaluation method is recommended. The following protocol, on how to analyse products for ST 131 contamination is based on found literature, with modification to suit a general foodborne isolation. It is a qualitative rather than quantitative approach.

Homogenize 10 g of product with 100 ml of EE broth (BD, Franklin Lakes, USA). Incubate homogenized product for 24 h at 37°C. After incubation, streak 100 µl of the homogenate onto Brilliance ESBL agar (Oxoid, Hampshire, UK) and incubate the plate for 24 h at 37°C (Geser et al., 2012). Using 1 ml of 0.9 % NaCl solution, dissolve all colonies and transfer the solution in a 1 ml tube. Dilute bacterial solution 1:10 using sterile water.

DNA extraction of all cells is performed via heat based lysis. Place the vial containing the solved and diluted bacterial mater in a heating block or similar at 95°C for 10 minutes. Centrifuge tubes at 10’000 x g for 1 minute and transfer the supernatant to a new tube.

Analysis of DNA is performed via standard PCR and screens only for ST 131, not any other factors such as ESBL, ExPEC or subclades. After the PCR, the obtained product is evaluated via electrophoresis on agarose gel or any other suitable method. All relevant settings for the PCR can be seen in the following tables. Any suitable ST 131 strain can be used as positive control for the PCR. A flowsheet of this described identification protocol can be seen in Figure 6.

*Table 5: Primers for ST 131 PCR.*

| Primer | Sequence | Size (bp) | Source |
|---|---|---|---|
| ST131_fw | GAC TGC ATT TCG TCG CCA TA | 310 | Doumith et al., 2015 |
| ST131_rev | CCG GCG GCA TCA TAA TGA AA | | Doumith et al., 2015 |

*Table 6: Amounts and components per sample/tube.*

| Component | Concentration | Amount | Final C |
|---|---|---|---|
| PCR Master Mix | 2 x | 10 µl | 1 x |
| Primer Forward | 10 µM | 0.8 µl | 0.4 µM |
| Primer Reverse | 10 µM | 0.8 µl | 0.4 µM |
| Template | - | 2 µl | - |
| $ddH_2O$ | - | Fill to 20 µl | - |

*Table 7: PCR cycler settings, source: (Doumith et al., 2015).*

| Step | | Settings |
|---|---|---|
| Heating lid | | 94°C, - |
| Initial denaturation | | 94°C, 3 min |
| Cycle x 30 | Denaturation | 94°C, 30 sec |
| | Annealing | 60°C, 30 sec |
| | Elongation | 72°C, 30 sec |
| Final Extension | | 72°C, 5min |
| Storage | | 8°C, ∞ |

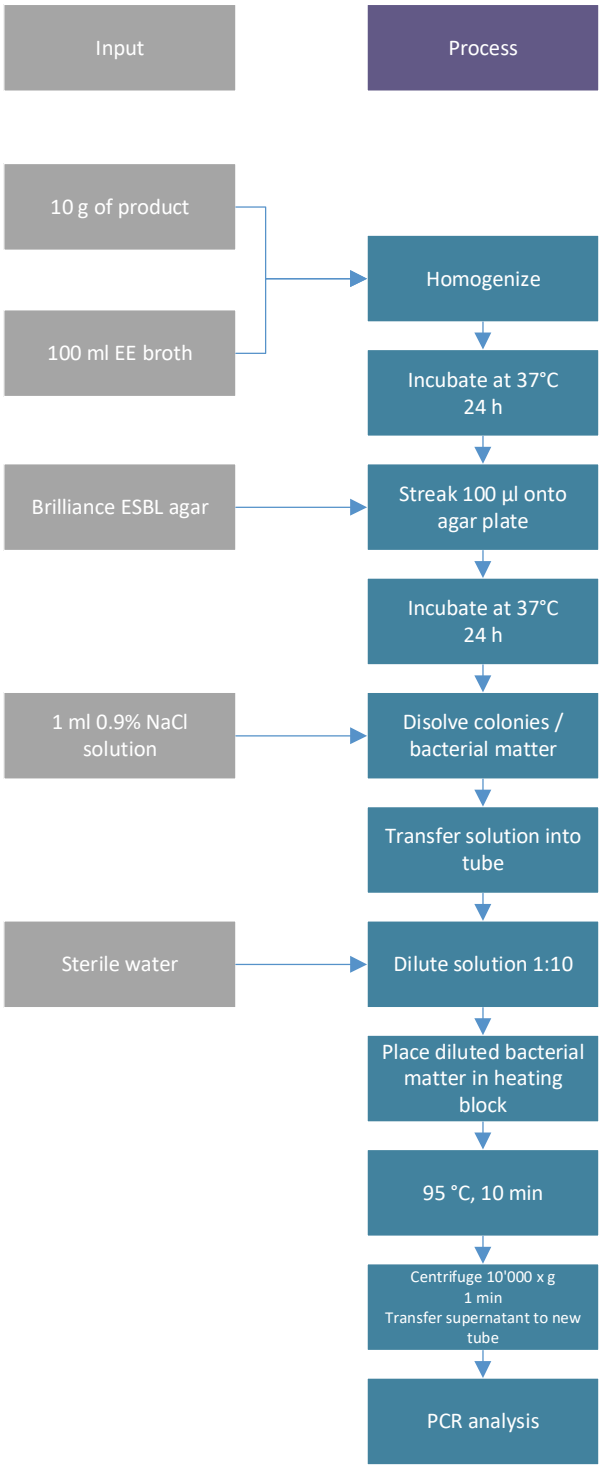


*Figure 6: Process flow sheet ST 131 identification from food samples.*