Zürcher Hochschule
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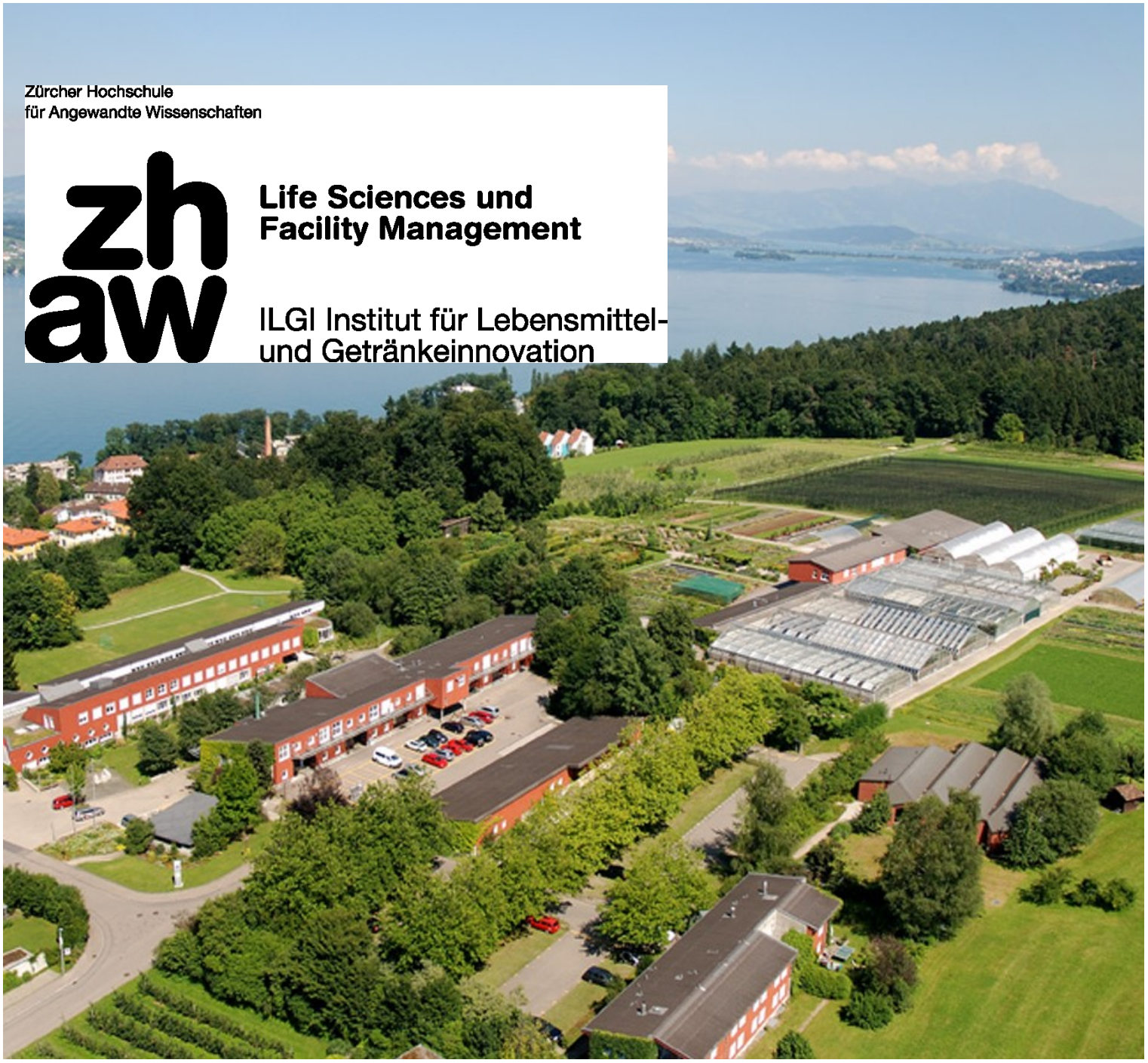

Prevalence of *E. coli* Sequence Type 131 as a foodborne pathogen in Swiss chicken

Report

by

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Abstract

E. coli Sequence Type 131 is an uropathogenic, multidrug resistant *E. coli* lineage. Within recent years, it has been suspected, that infections of ST 131 may occur as the result of contaminated food. During the previous report on this topic ("Risk Evaluation of E. coli Sequence Type 131 as a foodborne pathogen in Switzerland"), we outlined the potential threat ST 131 poses to the Swiss population. Results of this literature review showed, that ST 131 occurs frequently in chicken and poultry. This report shows the result of a prevalence test, conducted on Swiss chicken in the summer of 2020. A total of 200 samples were bought in retail stores in Wädenswil, Switzerland. After selective plating, samples were analyzed via PCR, targeting a ST 131 specific region. Out of the 200 samples analyzed, 25 (12.5 %) were tested positive. Further, samples were divided into organic and conventionally raised chicken. Organic samples (60, 33%) were tested positive in 12 cases (20 % of samples), meanwhile conventional samples (140, 66%) tested positive in 13 cases (9.2 % of samples). These results are slightly higher than expected based on literature research (5-10 %). Notably organic samples were comparatively high, with a contamination rate of 20 %.

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Abbreviation	Description
ESBL	Extended-Spectrum β-Lactamase
ST	Sequence Type
ExPEC	Extraintestinal Pathogenic
PCR	Polymerase Chain Reaction

1 Introduction

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 was thus tasked with reviewing available literature and evaluating the risk that this newly emerging threat poses to the Swiss population. After reviewing literature and doing a risk evaluation, the result was, that mainly chicken and poultry products are affected. Based on these findings, the ZHAW was further tasked with doing a prevalence test on these products, in order to confirm what was speculated based on literature. The following report details this prevalence test, and aims to answer the following questions.

- How high is the contamination rate of ST 131 in Swiss chicken, based on 200 samples?
- Do these findings conform to suggested rates based on literature?
- Is there a significant difference in contamination rate between organic and conventionally produced chicken?

This report is based on literature and methods described in the previous document “Risk assessment of ST 131 as a foodborne pathogen in Swiss food”. Necessary background information on ST 131 itself can also be found within.

2 Material & Methods

The following chapter describes all implemented methods and used materials & samples.

2.1 Samples

Retail chicken samples were bought in retail stores in Wädenswil, Switzerland. Samples were store bought and directly brought to the laboratory, where analysis started within 1 hour after buying. Samples bought included only raw, fresh chicken meat and only Swiss products were included. This, since buying either pre-cooked products such as sausages or chicken nuggets would not be comparable to fresh chicken when it comes to potential contamination. Further, it was a requirement by design of the prevalence test, to only include Swiss products. Samples were bought randomly, and aimed to include a large variety of different cuts, ranging from ground meat, minced, whole cuts such as breasts, up to samples with bones, such as wings or whole chickens.

2.2 Method

Samples were processed directly after buying. 10 g of sample, including bones, skin, meat and connective tissue, if applicable, were mixed with 90 ml of Enterobacteriaceae Enrichment broth (Oxoid, Hampshire, UK). Mixtures were homogenized in a stomacher bag for 1 minute at medium speed. After homogenization, samples were incubated at 37° C for 24 ± 4 h. Following incubation, 100 µl of each stomacher bag were streaked onto Brilliance™ ESBL agar (Oxoid, Hampshire, UK) and incubated for 24 ± 4 h at 37° C. The incubated plates were first evaluated visually for bacterial growth. Using 1.5 ml of sterile 0.9 % NaCl solution, all bacterial matter was suspended. In case of high bacterial growth, suspensions were diluted 1:10 using sterile H_2O. Cell suspensions were then placed in a heating block at 95° C for 10 minutes, followed by centrifuging at 10'000 x g for 2 minutes. Supernatants were transferred to a new tube and pelletized cells were discarded. Cell free supernatants were stored at -20° C. Evaluation for ST 131 was performed via PCR, using the supernatants as DNA templates. Primers and PCR settings can be seen in tables 2-5. Following PCR, products were evaluated via gel electrophoresis using a 1.2 % agarose gel at 90 V for 33 minutes. Figure 1 depicts an example agarose gel of the PCR process. Positive controls of ST 131 were obtained from the Vetsuisse Faculty, University of Zurich, Switzerland. Three different negative controls are in form of non-ST131 *E. coli* strains.

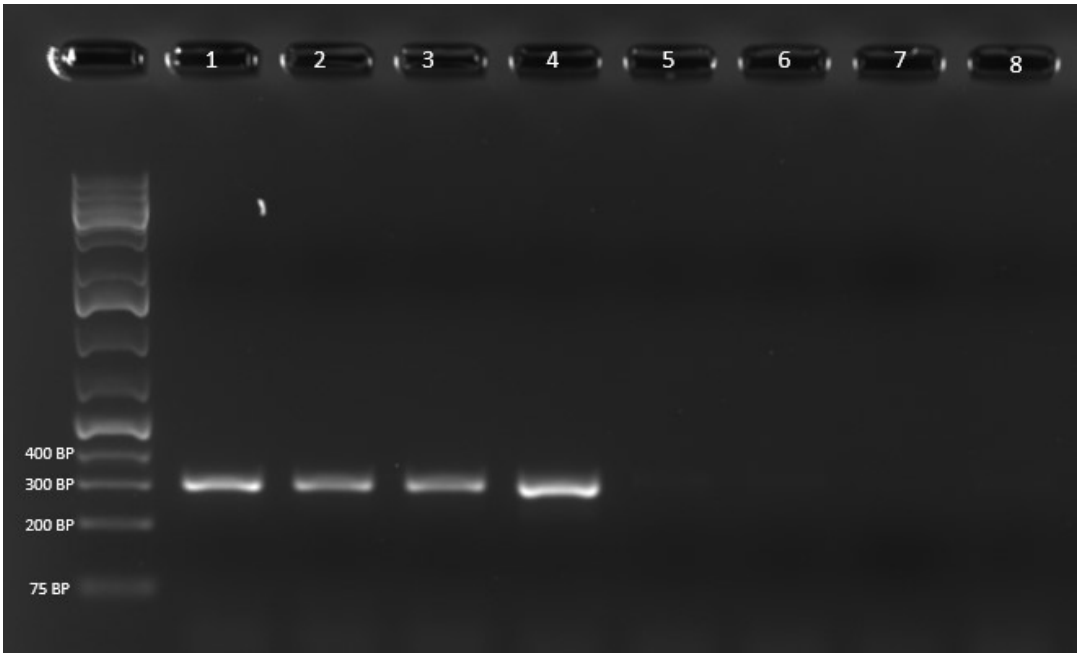

Figure 1: ST 131 example gel, 1: E. coli ST 131 DR06 - 2: E. coli ST 131 HC171 - 3: E.coli ST 131 OW18 - 4: Sample M4.2 (this study) - 5: E. coli STEC C600 - 6: E. coli ATCC 4157 - 7: E. coli ATCC 8739 - 8: ddH_20, negative control

Table 1: Primers 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 2: Components & concentrations ST 131 PCR

Component	**Concentration**	**Amount**	**Final C**
KAPA Taq ReadyMix	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 3: PCR cycler steps ST 131 PCR

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, 5 min
Storage		8° C, ∞

3 Results & Discussion

Across all 200 analyzed samples, a total of 25 (12.5 %) were tested positive. Looking at samples labelled as organic, accounting for 60 samples, 12 (20%) samples were tested positive (Figure 2a). The remaining 13 (9.2 %) positive tests originate from conventional or non-organic samples, accounting for 140 samples total (Figure 2b).

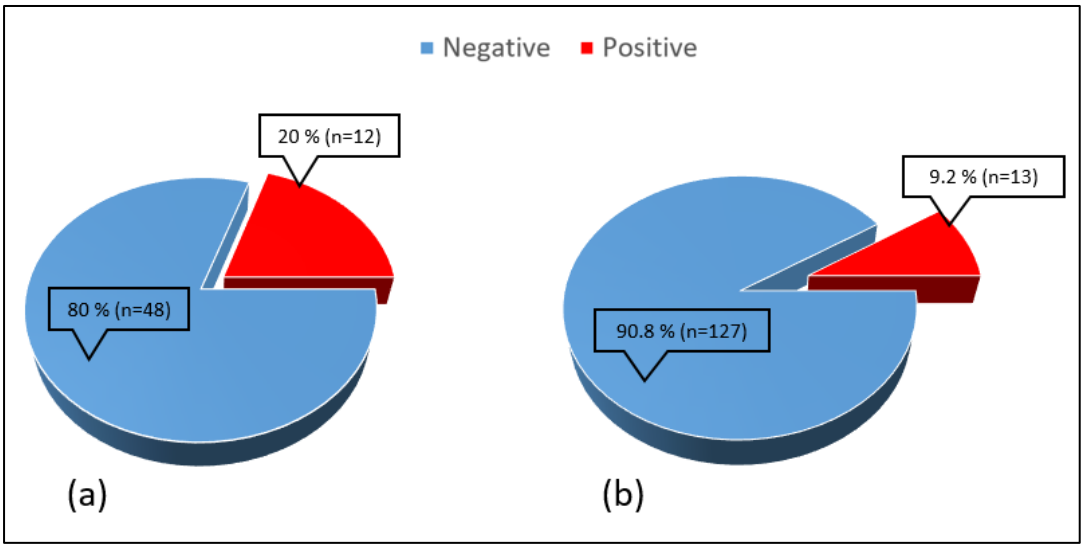

Figure 2: Positive samples for ST 131 with (a) positives in organic (20%, n=12) and (b) positives in conventional (9.2%, n=13)

Looking at these results, and comparing them with results obtained during the literature research, available in the previously mentioned risk-assessment, a few observations can be made. According to the literature research, it was expected, that between 5 to 10 % of samples will be contaminated and thus test positive for ST 131. In this study, across all samples, 12.5 % were tested positive, a slight increase towards the expected value. Within this study however, a clear trend is visible. Organic samples were tested positive twice as frequent compared to conventional samples (20% vs 9.2 %). Statistical significance was calculated in R Studios. Using Pearson's chi-squared test (without Yate's correction for continuity, as sample size is > 5), p-value at 95 % confidence interval was calculated as 0.035 and thus suggesting significant statistical difference between organic and conventional samples.

Looking at the comparison between different types of sample (whole cuts, ground, minced etc.) no statement can be made. Since these differences were not an aspect of this study, samples were not bought to account for it.

4 Literature

Doumith, M., Day, M., Ciesielczuk, H., Hope, R., Underwood, A., Reynolds, R., Wain, J., Livermore, D. M., & Woodford, N. (2015). Rapid Identification of Major Escherichia coli Sequence Types Causing Urinary Tract and Bloodstream Infections. *Journal of Clinical Microbiology*, *53*(1), 160–166. https://doi.org/10.1128/JCM.02562-14