ARAMIS-Nr. 4.23.05: Antimikrobielle Resistenzen in pflanzlichen Lebensmitteln (AMR-Green2)

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Aim of the study

The main objective of this project was to develop a protocol for monitoring antibiotic resistant-bacteria (ARB) and antibiotic resistance genes (ARGs) in fresh produce. A second aim was to investigate potential correlations between the prevalence of ARGs and ARB, to elucidate the use of ARGs as indicators of ARB load. In addition, a comprehensive literature review was done to summarize current knowledge on how post-harvest treatments may influence the dissemination of pathogens and antimicrobial resistance (AMR) in fresh produce.

Material and methods

Between summer 2023 and autumn 2024, a total of 150 fresh produce samples including tomatoes, carrots, lettuces, parsley and basil (50% locally produced and 50% imported) were purchased every second week from the 2 retailers that provide 80% of fresh produce sold in Switzerland. To minimize variability and cross-contamination, only conventionally grown (non-organic) and pre-packaged items were selected. Once in the lab, approximately 50 gr of each sample were aseptically weighed, diluted in 100 ml of 0.1% buffered peptone water and homogenized using Stomacher. The resulting homogenate served as starting material for the following 3 downstream analyses:

1) Prevalence of ARB: Prevalence of third-generation cephalosporin-resistant Enterobacterales (3GCR-E) and vancomycin-resistant Enterococci (VRE) was determined by direct plating of 10-fold serial dilutions.
2) Quantification of ARGs: DNA was extracted from the homogenates and analysed by quantitative PCR (qPCR) to quantify selected ARGs.
3) Isolation and Characterization: ESKAPEE-resistant pathogens were isolated and characterized after plating specific enrichments.

Results and significance

1) **Prevalence of ARB**
   3GCR-E were more prevalent in basil, parsley and lettuce (average of 4, 3.5 and 2.5 logCFU/g, respectively), compared to tomatoes and carrots, where 3GCR-E were barely isolated. VRE were isolated in some samples of carrot and parsley, not reaching an average of 1 logCFU/g.
2) **Occurrence of ARGs**
   Quantification of ARGs in the total DNA from bacteria present on the surface of fresh produce revealed that 87% of the samples contained at least one ARG, ranging from 97% in lettuce to 80% in carrots. Most of the detected genes conferred resistance to tetracyclines (*tetG*) and aminoglycosides (*aph3-Ib*). Notably, the *crAss*56 gene, that is a highly specific marker for human fecal contamination (Karkman et al., 2019) was detected in only a few carrot samples, suggesting potential contamination, likely from irrigation water. Fortunately, this occurred in only 5 out of the 150 tested samples.
3) **Molecular markers to track AMR in fresh produce**
   In leafy products (basil, parsley and lettuce), where the prevalence of ARB was higher, positive correlation of relative abundance of 3GCR-E with the genes *sul1* (resistance to sulfonamides) and *aadA2* (streptomycin) was determined. Therefore, they could be good candidates to trace AMR in fresh produce.
4) **Swiss vs. imported products**

Not significant differences were found between Swiss and imported fresh produce in terms of prevalence of ARB and ARGs. The large variation in the origins of the "imported" samples made it difficult to achieve a sufficient sample size with enough statistical power to allow comparisons between countries.

5) **Characterization of isolated WHO priority pathogens**
Presumptive 3GCR-E such as *Enterobacter* spp. and *Klebsiella* spp,, VRE and carbapenem-resistant *Pseudomonas aeruginosa*, all classified as ESKAPEE bacteria, were the most isolated bacteria in all types of analyzed fresh produce. Other 3GCR-E such as *E.coli*, *Citrobacter* spp., *Morganella morganii, Raoultella* spp. and *Serratia* spp. were isolated in some types of produce. A selection of isolates with interesting resistance phenotypes (n=34) were sequenced using long-read technology (Nanopore). The screening of the genomes using the AMR Finder tool showed that almost all the Enterobacterales carried a gene conferring resistance to cephalosporins, as expected, as they were isolated in a media containing cefotaxime. Besides, they harboured a variety of genes conferring resistance to aminoglycosides, beta-lactams, colistin and tetracycline, among others. The strains harbouring *sul1*, mainly found in plasmids, commonly harboured *aadA*. This fact is noteworthy as these 2 genes were also mentioned before as good molecular markers for the presence of 3GCR-E in a sample. Three *Enterobacter* spp. isolates from tomatoes and basil, harboured *mcr* genes in a plasmid, conferring resistance to colistin, a last-resort antibiotic used to treat infections caused by multidrug-resistant Gram-negative bacteria. Fortunately, genes conferring resistance to carbapenems, another class of last-resort antibiotics, were detected only in three *Pseudomonas aeruginosa* isolates, all obtained from basil. These genes are intrinsic to the bacterial chromosome and confer only low-level carbapenem resistance. One *E. coli* isolated from basil, hosted one plasmid containing the $bla_{\text{CTX-M-15}}$ gene, one of the most widespread and clinically significant ESBL gene conferring resistant to beta-lactams and cephalosporins.

6) **Review about post-harvest treatments** and their influence on the dissemination of pathogens and antimicrobial resistance (AMR) in fresh produce (Find attached as "Review_postharvest_AMR_V5).

These results, together with the AMR_Green1 outputs, indicate that fresh produce, particularly herbs, serve as important reservoirs of ARB and ARGs, including resistant ESKAPEE pathogens. On the other hand, given that fresh produce harbor clinically relevant ARGs that may be ultimately transferred to the human gut microbiome upon ingestion, continued monitoring is essential. In this study, the initial pre-screening of the *sul1* and *aadA2* genes in total DNA from the samples proved to be a useful approach to identify those more likely to carry ARB, reinforcing its role as an AMR molecular indicator. Moreover, screening for *sul1* in bacterial isolates can provide additional information into which isolates harbor multiple resistance determinants. These results are in line with previous findings, as *sul1* was already suggested as good indicator for the total abundance of ARGs in a fresh produce (Klaui et al.,2024) and it has been suggested by many authors as an indicator of anthropogenic contamination in waters soils, and sediments (Berendonk et al., 2015). In conclusion, although food microbiology is still based on traditional techniques, the combination with qPCR and WGS can provide new insights to monitor AMR in food samples.

Publications, posters and presentations

- Oral presentation in the 4. Microbiological Risk Assessment (MRA) Seminar, "*Antimicrobial resistance in plant-based foods",* Lisa Thönen.
- Scientific publication, original research article: "*Herbs as hotspots for AMR spread*". In preparation
- Scientific publication, review: "*Evaluating the Contribution of the post-harvest treatments on the spread of Antimicrobial Resistance on fresh produce".* Annexed to this report.

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Bibliography

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