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RESEARCH ARTICLE

Studies on uptake and distribution of antibiotics in red cabbage

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Abstract A large source of antibiotic active compounds and resistant bacteria is livestock waste, distributed as fertilizer on fields, used for vegetable cultivation. Frequently consumed vegetable, contaminated by low levels of antibiotics, may contribute to the development of bacterial antibiotic resistance. The objective of this work was to estimate the possible role of red cabbage as reservoir and carrier of antibiotic contaminants into the food chain. Red cabbage was exposed to antibiotics in hydroponic cultures and farming conditions. Enrofloxacin (ENR), chlortetracycline (CTC), monensin (MON) and amoxicillin (AMO) were separately added to nutrient solutions. Analysed by sequential extraction and LC–MS/MS methods, the plants revealed a remarkable uptake of CTC and in particular ENR (old leaves 0.22 mg/kg fresh weight (fw) CTC, 6.0 mg/kg fw ENR; roots up to 215.0 mg/kg fw CTC, 14.6 mg/kg fw ENR). The uptake of MON was significantly lower and AMO was not detectable. Red cabbage was also grown on manure-fertilized plots. The pig manure contained defined amounts of CTC and ENR (50 and 150 mg/kg). At harvest the edible parts of cabbage had between 9.2 to 16.9 μg/kg fw ENR, while there was no evidence obtained for the uptake of CTC. Finally, production steps of canned red cabbage were examined for carry over effects. Traces of tetracycline (16.4–19.2 μg/kg fw) were detected in supplies of freshly harvested vegetable, grown conventionally, but not in the marketable final product.

Keywords Plant uptake · Antibiotics · Red cabbage · Hydroponic · Field experiments · Carry-over

1 Introduction

It is quite clear that veterinary antibiotics have benefits for animal health and also risks due to enrichment of resistant bacteria (Smalla et al. 2014). Recognizing the importance of increasing resistance, the WHO summarizes the prevalence of drug resistance worldwide in a "Global Report of Surveillance 2014" and warns of an era of deadly infections (WHO 2014). In May 2015, the WHO issued a "Global Plan of Action to combat antibiotic resistance", considering animal health and global food security (Federal Ministry of Health 2014). The Federal Government of Germany addressed this problem by launching in November 2008 "DART" (Deutsche Antibiotika-Resistenz-Strategie) which aims to reduce the formation and spread of antibiotic resistance (DART 2011, 2015).

Since 2011, pharmaceutical companies and wholesalers in Germany are obliged to report the amount

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of antibiotics supplied to veterinaries according to a regulation of DIMDI (Deutsches Institut für Medizinische Dokumentation und Information). With the aim of reducing antibiotics in livestock, the German Drug Law has been amended in July 2014. Antibiotic treatments on animal farms are collected in a database for benchmarking (Arzneimittelgesetz 2005).

Livestock waste which is distributed on fields as fertilizer is a potentially large source of antibiotics. In former investigations, transfer of antibiotics from slurry fertilized soil in vegetables was demonstrated (Kumar et al. 2005; Boxall et al. 2006; Dolliver et al. 2007; Freitag et al. 2008; Grote and Heyser 2007; Grote et al. 2006, 2007a, b, 2009; Maia et al. 2009; Marsoni et al. 2014; Goldstein et al. 2014).

The role of vegetables as a reservoir and carrier of antibiotic contaminants requires clarification, because it has been shown that antibiotic concentrations far below the minimum inhibitory concentration can select for antibiotic resistance in bacteria (Sandegren 2014). Thus, it cannot be excluded that low level concentrations of antibiotics in food plants contribute to the development of bacterial antibiotic resistance.

In order to assess possible consequences with regard to food safety, in our study, red cabbage, a frequently consumed vegetable was tested for its ability to take up highly prescribed veterinary drugs under hydroponic conditions as well as under farming conditions.

Furthermore, the production steps of canned red cabbage were examined for carry over effects of possible antibiotic contaminants.

2 Methods and materials

2.1 Experiments with hydroponic cultures

Red cabbage plants were grown in nutrient solution and exposed to selected representative antibiotics: amoxicillin (AMO), chlortetracycline (CTC), monensin (MON) and enrofloxacin (ENR). Seeds were germinated in calcium sulfate solution to seedling stage with first leaves. Seedlings were transferred to pots filled with 4.5 L of modified Hoaglands nutrient solution (pH 6), which were placed in a climate-controlled growth cabinet applying a 16 h photoperiod (Grote et al. 2009). The nutrient solution was exchanged 3 times a week. Investigations of spiked nutrient solution showed that the antibiotics were stable even 3 days after spiking. Because methanol was used for the preparation of the AMO stock solution, formation of an amoxicillin-methanol adduct was observed, as reported already (Grujic et al. 2008). After 14 days, the nutrient solution was spiked with either AMO, CTC, MON or ENR, so that concentrations of 5.0 (series A) or 2.5 μmol/L (series B) were reached for each compound. Non-spiked nutrient solution was used as negative control. The plants were harvested 14 days after spiking and roots were intensively rinsed. Roots and stems, young and old leaves, were separated and stored at −60 °C.

2.2 Field experiments

The field experiments under controlled farming conditions were conducted at the "Versuchszentrum Gartenbau in Köln-Auweiler" (Chamber of agriculture NRW, Germany). No manure had been applied to these fields for the last 10 years and no cultivation was carried out in that period. The composition of this soil "sandy, silty loam" [43.2 % silt, 38.7 % sand and 18.1 % clay, pH ($CaCl_2$): 6.4] is typical for this area in North Rhine Westphalia. Liquid manuring was performed using pig slurry, provided by the Federal Research Institute for Animal Health (Friedrich-Löffler-Institut) in Braunschweig, Germany. This initially antibiotic free pig slurry was spiked prior to spreading onto the experimental plots with CTC and ENR. These antibiotics had been chosen because of their high uptake into red cabbage under hydroponic conditions.

Based on the cropping plan, the experimental plots were arranged in order to get 4 variations of fertilization and 3 repetitions each. The variations were based on mineral fertilization, organic fertilization with antibiotic free slurry and with CTC and ENR spiked slurry in 2 concentrations (50 and 150 mg/kg). After fertilization and tilling, seedlings of red cabbage (*Brassica oleracea var. capitata f. rubra),* type "Rodon" were planted manually in soil blocks (50 days after germination). Slurry was sampled before manure application, soil samples (plough layer, 0–30 cm) were collected before and after organic fertilization and during harvest. The harvested plants were separated into edible parts, outer leaves and roots and stored at −60 °C.

2.3 Residue analysis in running red cabbage canning production

A large percentage of red cabbage production is on an industrial basis, where the final product is canned. Therefore, an additional study was carried out in cooperation with a food company in Germany, which

manufactures canned red cabbage. Residue analyses were carried out to detect carry-over effects of antibiotics as possible contaminants in conventionally cultivated (contract farming) raw red cabbage, in various production stages and in the final sale product. More specifically, during the running production, samples were taken from stalks, outer leaves, cabbage after slicing, steaming, before pasteurization and the canned marketable product.

2.4 Analysis of antibiotic residues

Sample materials like spiked animal excrements, soil and plants that were exposed to antibiotics under farming and hydroponic conditions were analysed for extractable amounts of CTC, ENR, AMO, MON and some of their main metabolites or conversion products (e.g. iso-chlortetracycline (iso-CTC), tetracycline (TC), demeclocycline (DMC) and epimers; ciprofloxacine (CIP), AMA: amoxicilloic acid and Diketo: amoxicillin diketo-piperazine-2′,5′-dione).

The analytical methods are based on ultrasonic assisted extraction, clean-up procedures, e.g. solid phase extraction (SPE) and LC–ESI–MS/MS determination. The methods, already described for the analysis of white cabbage and other crops (Grote et al. 2006, 2009; Choi et al. 2014), were adapted to red cabbage organs (roots, stems, inner and outer leaves). Details of the method development and validation data elaborated by Chowdhury (2012) are available in supplementary materials.

By means of KH_2PO_4-solution (pH 2.0) the extraction yields obtained for edible parts of cabbage reached from 93 % for CTC, 99 % for ENR, 90 % for CIP to 45 % for MON. The determination of AMO and its metabolites revealed certain problems due to the rapid conversion of these compounds in contact with plant materials and formation of solvent adducts in the ESI-ion trap system, which caused relatively low recovery values. However, recovery values from spiked buffer solution were 56 % for AMA, 77 % for AMO and 91 % for Diketo. The identification of conversion and decomposition products of ß-lactams in soil and manure was object of detailed investigations (Michels et al. 2013).

Published methods (Turiel et al. 2006; Karci and Balcioglu 2009; Sturni et al. 2012; Grote et al. 2006, 2007b, 2010) were optimized for efficient extraction of administered antibiotics from soil of the experimental fields, e.g. fluoroquinolones ($Mg(NO_3)_2/NH_3$ extraction: ~39 % ENR) and chlortetracyclines (NH_3/NH_4Cl/EDTA extraction: 75 %).

3 Results and discussion

3.1 Experiments with hydroponic cultures

Spiking experiments in hydroponic cultures have the advantage that no sequestration of antibiotics on soil particles can occur. Thus, this experimental system is well suited to initially analyse plant capacity for uptake of a certain antibiotic.

The results of the uptake experiments (Tables 1, 2) reveal that CTC and ENR are preferably taken up from the nutrient solution by red cabbage. Maximum values of CTC loading (sum of CTC and iso-CTC, a conversion product, including tautomers and epimers) were measured in roots (spiking 2.5 μmol/L: ~76 mg/kg fw; 5.0 μmol/L: 215 mg/kg fw). Obviously, doubling the spiking concentration results in a threefold uptake of CTC. Thus, an increasing concentration of CTC leads to an increased concentration in plant tissue. The evidence of CTC-compounds in stems (148 μg/kg fw or 402 μg/kg fw) and leaves (young leaves 174 μg/kg fw; old leaves 215 μg/kg fw) indicates that a transfer in the plants had taken place. The concentrations of CTC in old leaves (215 μg/kg fw) differ only slightly from young leaves (174 μg/kg fw, Table 2). This phenomenon was also observed in white cabbage (Grote et al. 2009). Furthermore, traces of TC, epi-TC (e-TC), doxycyclin (DC), demeclocyline (DMC) and its epimer (e-DMC) were detected (Tables 1, 2), which were described in former studies as conversion or decomposition products ("metabolites") of CTC (Grote et al. 2006, 2007a, 2009).

The plants grown in ENR spiked nutrient solution developed faded, yellow leaves. This effect may be attributed to the inhibition of photosynthesis by the fluoroquinolone (Aristilde et al. 2010). It was striking that these leaves were much larger compared to the green leaves of the control plants. Such effects of antibiotics on plant growth were described in former studies (Jjemba 2002), also for white cabbage (Grote et al. 2009).

The ENR concentrations varied to some extend in different plant organs (young leaves, old leaves, stems and roots), however ENR concentrations reached the same levels in stems and roots, independent of the spiking concentration (Tables 1, 2). Of all organs, roots contained highest ENR levels at approximately 15 mg/kg fw and stems at 2.4 mg/kg fw. Also, the ENR concentrations in old leaves and young leaves did not differ much within the 2.5 μmol/L (each ~3 mg/kg) or 5.0 μmol/L (each

Table 1 Uptake of antibiotics into red cabbage in hydroponic culture (2.5 μmol/L spiking-concentration)

Spiking with	Sample	AMO	AMO-MeOH	AMA	Diketo	CTC	epi-keto-CTC	epi-enol-CTC	iso-CTC	epi-iso-CTC
AMO	AMO-OL	–	–	–	–	–	–	–	–	–
	AMO-YL	–	–	–	–	–	–	–	–	–
	AMO-S	–	–	–	–	–	–	–	–	–
	AMO-R	–	–	–	–	–	–	–	–	–
CTC	CTC-OL	–	–	–	–	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	CTC-YL	–	–	–	–	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	CTC-S	–	–	–	–	41.4 μglkg	5.3 μg/kg	94.4 μg/kg	6.7 μg/kg	–
	CTC-R	–	–	–	–	*19.0 mg/kg*	*36.1 mg/kg*	*19.8 mg/kg*	898.7 μg/kg	274.2 μg/kg
ENR	ENR-OL	–	–	–	–	–	–	–	–	–
	ENR-YL	–	–	–	–	–	–	–	–	–
	ENR-S	–	–	–	–	–	–	–	–	–
	ENR-R	–	–	–	–	–	–	–	–	–
MON	MON-OL	–	–	–	–	–	–	–	–	–
	MON-YL	–	–	–	–	–	–	–	–	–
	MON-S	–	–	–	–	–	–	–	–	–
	MON-R	–	–	–	–	–	–	–	–	–

Spiking with	TC	epi-TC	DMC	DC	ENR	CIP	MON
AMO	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
CTC	<LOQ	<LOQ	–	–	–	–	–
	<LOQ	<LOQ	–	–	–	–	–
	<LOQ	–	–	48.0 μg/kg	–	–	–
	1.0 mg/kg	*1.3 mg/kg*	74.2 μg/kg	–	–	–	–
ENR	–	–	–	–	*2.4 mg/kg*	<LOQ	–
	–	–	–	–	*3.1 mg/kg*	<LOQ	–
	–	–	–	–	*2.4 mg/kg*	32.0 μg/kg	–
	–	–	–	–	*14.4 mg/kg*	<LOQ	–
MON	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	<LOQ
	–	–	–	–	–	–	<LOQ

Sample-weight: 5 g homogenate (leaves and stems), 3 g (roots); *YL* young leaves, *OL* old leaves, *S* stems, *R* roots

Results expressed as *mg/kg* or μg/kg fresh weight (fw homogenate); values are means from 2 samples, 3 measurements of each by HPLC–MS/MS, matrix calibration, LOQ: limit of quantification, values are defined in supplementary materials

~6.5 mg/kg) series. These observations suggest that roots and stems of the cabbage plant absorbed ENR until a saturation of ENR was reached, relatively independent of the supply in the nutrient solution in which it was grown. However ENR-concentrations in leaves were almost twice as high, when spiking increased from 2.5 to 5.0 μmol/l ENR in nutrient solution. Thus, on the basis of the available data, it cannot be decided for the whole cabbage plant, whether a saturation level of ENR accumulation has been reached. If a saturation phenomenon is occurring for ENR, it must be subject to certain substance specificity, because it could not be observed in the case of CTC. It is of interest to note that almost identical ENR levels occurred in different organs of white cabbage, which was cultivated under similar conditions (Grote et al. 2009).

CIP, the conversion product of ENR, detected in all plant organs (<LOQ), was measured only in the stems (32 or 43 μg/kg fw).

In contrast to ENR, MON has a growth-inhibiting influence on red cabbage. Only in MON treated

Table 2 Uptake of antibiotics into red cabbage in hydroponic culture (5.0 μmol/L spiking-concentration)

Spiking with	Sample	AMO	AMO-MeOH	AMA	Diketo	CTC	epi-keto-CTC	epi-enol-CTC	iso-CTC	epi-iso-CTC
AMO	AMO-OL	–	–	–	–	–	–	–	–	–
	AMO-YL	–	–	–	–	–	–	–	–	–
	AMO-S	–	–	–	–	–	–	–	–	–
	AMO-R	–	–	–	–	–	–	–	–	–
CTC	CTC-OL	–	–	–	–	157.6 μg/kg	<LOQ	<LOQ	57.6 μg/kg	<LOQ
	CTC-YL	–	–	–	–	102.2 μg/kg	<LOQ	<LOQ	71.7 μg/kg	<LOQ
	CTC-S	–	–	–	–	154.0 μg/kg	52.7 μg/kg	164.2 μg/kg	30.9 μg/kg	<LOQ
	CTC-R	–	–	–	–	*60.8 mg/kg*	*94.6 mg/kg*	*55.7 mg/kg*	*2.8 mg/kg*	*1.0 mg/kg*
ENR	ENR-OL	–	–	–	–	–	–	–	–	–
	ENR-YL	–	–	–	–	–	–	–	–	–
	ENR-S	–	–	–	–	–	–	–	–	–
	ENR-R	–	–	–	–	–	–	–	–	–
MON	MON-OL	–		–	–	–	–	–	–	–
	MON-YL	–	–	–	–	–	–	–	–	–
	MON-S	–	–	–	–	–	–	–	–	–
	MON-R	–	–	–	–	–	–	–	–	–

Spiking with	TC	epi-TC	DMC	epi-DMC	ENR	CIP	MON
AMO	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
CTC	<LOQ	<LOQ	–	–	–	–	–
	<LOQ	<LOQ	–	–	–	–	–
	12.9 μg/kg	–	–	–	–	–	–
	3.5 mg/kg	*3.9 mg/kg*	340.2 μg/kg	175.7 μg/kg	–	–	–
ENR	–	–	–	–	*6.0 mg/kg*	< LOQ	–
	–	–	–	–	*7.0 mg/kg*	<LOQ	–
	–	–	–	–	*2.4 mg/kg*	42.7 μg/kg	–
	–	–	–	–	*14.6 mg/kg*	<LOQ	–
MON	–	–	–	–	–	–	<LOQ
	–	–	–	–	–	–	<LOQ
	–	–	–	–	–	–	<LOQ
	–	–	–	–	–	–	*1.58 mg/kg*

Abbreviations see Table 1

Results expressed as *mg/kg* or μg/kg fresh weight (fw homogenate); values are means from 2 samples, 3 measurements of each by HPLC–MS/MS, matrix calibration, LOQ: limit of quantification, values are defined in supplementary materials

plants a transparent, white substance concentrated around the leaf vasculature on the leaf surface. The macrolide was quantified only in the roots at 1.58 mg/kg fw (Table 2) whereas the transfer into other plant organs was rather low (<LOQ).

The detection of AMO in hydroponically grown red cabbage was not possible. As it metabolizes easily in the animal organism to AMA and Diketo (Reyns et al. 2008), plants were also analysed for these compounds. However, the detection of these possible metabolites of AMO was unsuccessful. In addition, in the presence of methanol, amoxicillin is able to form an AMO-MeOH-adduct (Grujic et al. 2008). Because methanol was used during sample preparation (SPE), the formation of the AMO-MeOH adduct was also investigated by LC–MS/MS. In fact, small traces were found in root samples but no traces in other plant tissues.

It is suspected that the degradation of AMO is accelerated by the release of enzymes out of normally separated plant compartments during sample preparation by grinding (homogenization) of the

plant organs. Other factors may play a role. For example, the incorporated antibiotics can be stored in the plant cell vacuole, where they can be degraded (Marty 1999; De 2000). The plants, grown in AMO spiked nutrient solution were not visibly different from the control plants, which may underline the hypothesis of plant detoxification mechanisms (Breuer 2001).

3.2 Field experiments

Red cabbage was grown in one cultivation period from spring to summer on experimental plots. The organic fertilization was performed with antibiotic free pig slurry (control plots) and with slurry containing both CTC and ENR, spiked at two concentrations: 50 and 150 mg/kg.

As expected, no antibiotic could be found in control plants, grown on plots receiving antibiotic free slurry (Table 3). ENR was detected in all organs of the cabbage plants which were grown on plots fertilized with ENR spiked manure (Table 3). Despite reports that fluoroquinolones, like ENR, are strongly sorbed in soil matrix (Leal et al. 2013), the substance was obviously bioavailable in these field experiments here. In edible parts of the vegetable concentrations of ENR ranged from 12.4 to 16.9 μg/kg fw in inner leaves to ~10 μg/kg fw in outer leaves and roots as well. The results indicate a relatively low influence of the ENR-spiking concentrations (1:3 ratio). Taken together with results from hydroponics for roots and stems the conclusion is that ENR was accumulated in the cabbage plants up to a certain saturation level. However, this hypothesis would need to be tested in further experiments.

Unexpectedly, the residue analysis did not provide the evidence for CTC, although the cabbage plants preferentially carried over CTC as well as ENR in the hydroponic experiments (Tables 1, 2). It is assumed that the particular sorption behavior of CTC in the soil matrix causes this effect. Tetracyclines are described as immobile and persistent (Thiele-Bruhn 2003). However, the absorption characteristic of CTC to the soil matrix may result in a drastic reduction of its bioavailability.

The uptake and distribution of pharmaceuticals in plants depends on various physical–chemical parameters such as lipophilicity and extent of ionization in the soil (Trapp 2004; Wu et al. 2011; Wegst-Uhrich et al. 2014; Carter et al. 2014). In particular, the sorption of CTC to soil particles is influenced by the pH dependent protolytic equilibrium of this tetracycline. Investigations by Thiele-Bruhn (2003) revealed that CTC in zwitterionic form is strongly adsorbed by a slightly acidic soil. The pH-value of the soil of the experimental plots was between 6.2–6.6. Under these conditions CTC is mainly present as a zwitterion, thus promoting its adsorption onto soil particles.

The analyses of soil extracts supported the hypothesis that pH conditions in the soil and during extraction conditions are important for the amount of detectable analytes.

To optimise the extraction procedure, soil samples were taken from field plots that had not received antibiotics through manure. These soil samples were freshly spiked at 50 mg/kg each for CTC and ENR. After suspension of these soil samples in NH_3/NH_4Cl/EDTA buffer (pH 10), the sum of CTC (∑CTC) was desorbed efficiently from the soil samples: ~75 % were recovered (Table 4). Similar recovery values were reported previously (Grote et al. 2006, 2007a). In contrast the yield for fluoroquinolones in this procedure was very low (<10 %, Table 4). Magnesium nitrate buffer (pH 8.5), is able to extract ENR and CIP from soil at higher rates (36–39 %), whereas the extractability of CTC is reduced to 34 % (Table 4).

It is striking that at the time of harvest, i.e. 3 months after manure application, the extractability of chlortetracycline from soil is rather low: only traces <5 μg/kg ∑CTC (spiking 50 mg/kg) and 9.9 μg/kg (spiking 150 mg/kg) (Table 5) could be found. In contrast, ENR maintained higher extractability levels: 15.1 μg/kg (spiking 50 mg/kg) and 51.5 μg/kg (spiking 150 mg/kg). Traces of CIP, arising from de-ethylation of ENR, were also detected in the soil (~3.1 μg/kg).

Considering the distinct sorption behavior of tetracycline in soil and disregarding chemical loss processes, it is feasible that the availability of CTC for the cultivated plants is low and, as a consequence, an uptake by the red cabbage negligible.

3.3 Red cabbage production

In order to detect carry-over effects of antibiotics as possible contaminants in conventionally cultivated raw red cabbage, residue analyses were carried out in various production stages up to the final sale product. During running production, samples were taken from stalks, outer leaves, cabbage after slicing, steaming, before pasteurization and the canned marketable product.

Red cabbage samples from three German suppliers were analyzed. Four pooled samples from one supplier showed antibiotic contamination. TC was found in outer leaves of raw cabbage (19.2 μg/kg fw) and

Table 3 Enrofloxacin residues in red cabbage grown on manure-applied soil

Plant organs	Variation 1 Mineral control fertilization	Variation 2 Organic control fertilization	Variation 3 Spiked manure 50 mg/kg	Variation 4 Spiked manure 150 mg/kg
Inner leaves	<LOD	<LOD	12.4 ± 1.8	16.9 ± 0.9
Outer leaves	<LOD	<LOD	10.9 ± 0.8	10.6 ± 0.5
Roots	<LOD	<LOD	9.2 ± 0.4	11.9 ± 1.0

1st manure fertilization: 25.05.2010; 93 kg N/ha: 223.2 g N/each plot (24 m^2); 60 kg manure/plot

1st mineral fertilization: 25.05.2010; 92 kg N/ha: calcareous ammon saltpetre (CAS; 27 % N), 3.06 kg/90 m^2

Planting: 25.05.2010 after fertilization

2nd fertilization (mineral, all plots): 28.06.2010: 101.0 kg/ha: 3.36 kg CAS on control plots, 133.5 kg/ha: 4.44 kg CAS on spiked manure plots

3rd fertilization (mineral, all plots): 11.08.2010: 101.0 kg/ha: 3.36 kg CAS on all plots

Sampling (harvest): 14.08.2010

ENR-uptake in (μg/kg fw); CTC was not detected in any sample

Results are mean ± SD (standard deviation) from 4 samples, 3 measurements of each by HPLC–MS/MS, LOD: limit of detection, values are defined in supplementary materials

Table 4 Recovery (%) of chlortetracycline and fluoroquinolones (ENR, CIP) from soil spiked with antibiotics

Extractant	∑CTC (iso-CTC)	ENR	CIP
NH_3/NH_4Cl/EDTA (pH 10)	75.1 ± 6.8	6.8 ± 1.0	6.8 ± 1.0
$Mg(NO_3)_2/NH_3$ (pH 8.5)	33.8 ± 7.6	38.8 ± 4.7	35.8 ± 6.2

Sample-weight: 5 g soil (air dried, control plots), spiking 50 mg/kg soil, 10 mL extractant, 30 min shaking

Results are mean ± SD from 3 samples, 3 measurements of each by HPLC–MS/MS

Table 5 Extractable amounts [μg/kg] of antibiotics and conversion products in soil at time of harvest of red cabbage

Antibiotic (conversion products) extractant	Variation 1 Mineral control fertilization	Variation 2 Organic control fertilization	Variation 3 Spiked manure 50 mg/kg	Variation 4 spiked manure 150 mg/kg
ENR $Mg(NO_3)_2/NH_3$ (pH 8.5)	<LOD	<LOD	15.1 ± 4.5	51.1 ± 16.4
CIP $Mg(NO_3)_2/NH_3$ (pH 8.5)	<LOD	<LOD	<LOQ**	3.1 ± 0.7 (≥LOQ**)
∑CTC (iso-CTC)* NH_3/NH_4Cl/EDTA (pH 10)	<LOD	<LOD	<LOQ**	9.9 ± 2.2

Results are mean ± SD from 2 samples, 3 measurements of each by HPLC–MS/MS

* CTC completely converted to iso-CTC (sum of epimers)

** LOQ (see supplementary materials)

also in sliced cabbage (16.4 μg/kg fw). Further sampling and subsequent residue analysis was performed after passing the vegetable through the 3 steaming units. Red cabbage was also analyzed before and after pasteurization. TC could not be detected in any of these production steps, and most importantly, also not in the final marketable product. This may be due to the fact that the residues of TC traces are degraded during the thermal processing steps. Under these conditions toxic anhydro-derivates of tetracycline may be formed (Kühne et al. 2001), which could, however not be detected. It may also be possible that

in the first steaming process TC migrates into the brine, which is discarded.

However, due to the non-representative small number of samples (12 pooled samples per supplier) definitive conclusions can not yet be drawn from these results to assess possible consequences with regard to food safety.

4 Conclusions

The results of this study give evidence, that red cabbage plays a role as possible reservoir and carrier of antibiotic contaminants into the food chain, such as tetracyclines and fluoroquinolones. We found that in hydroponic conditions old leaves contained up to 0.22 mg/kg fw CTC and 6.0 mg/kg fw ENR. Further increased, roots revealed up to 215.0 mg/kg fw CTC and 14.6 mg/kg fw ENR. AMO and possible conversion products could not be identified in plant samples, whereas traces of MON were quantified in roots (1.6 μg/kg fw) and recorded below LOQ in leaves.

Under controlled farming conditions, red cabbage plants take up ENR also from manure-fertilized plots by the roots and incorporate it into the edible parts (9.2–16.9 μg/kg fw). We emphasize that the pig manure was spiked with both, ENR and CTC. Contrary to the hydroponic experiments, CTC and conversion products were not detectable in any of the field samples, which might be predominantly an effect of strong sorption to soil matrix.

Anyway, traces of TC (16.4–19.2 μg/kg fw) were detected in supplies of freshly harvested vegetable, grown conventionally on an agro-industrial basis. Indications for carry over effects of tetracycline were not found in the production steps of canned red cabbage and, as a consequence, not in the marketable final product.

Obviously, red cabbage is a frequently consumed vegetable, can be a carrier of low levels of antibiotics into the food chain. Consumer antibiotic exposure through food of plant origin is most likely to occur. For food safety reasons, it needs to be investigated in further research, whether low levels of antibiotics in food plants can contribute to development of bacterial resistance. Among others, these objectives are pursued in the BMBF-supported interdisciplinary joint project RESET ("ESBL and fluoroquinolone resistance in *Enterobacteriaceae*", www.reset-verbund.de).

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