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Occurrence of antibiotic-resistant enterobacteria in agricultural foodstuffs

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Antibiotic-resistant bacteria or their corresponding resistance determinants are known to spread from animals to humans *via* the food chain. We screened 20 vegetable foods for antibiotic-resistant coliform bacteria and enterococci. Isolates were directly selected on antibiotic-containing selective agar (color detection). Thirteen "common vegetables" (tomato, mushrooms, salad) possessed $10^4 – 10^7$ cfu/g vegetable of coliform bacteria including only few antibiotic-resistant variants ($0 – 10^5$ cfu/g). All seven sprout samples showed a some orders of magnitude higher contamination with coliform bacteria ($10^7 – 10^9$ cfu/g) including a remarkable amount of resistant isolates (up to 10^7 cfu/g). Multiple resistances (up to 9) in single isolates were more common in sprout isolates. Resistant bacteria did not originate from sprout seeds. The most common genera among 92 isolates were: 25 *Enterobacter* spp. (19 *E. cloacae*), 22 *Citrobacter* spp. (8 *C. freundii*), and 21 *Klebsiella* spp. (9 *K. pneumoniae*). Most common resistance phenotypes were: tetracycline (43%), streptomycin (37%), kanamycin (26%), chloramphenicol (29%), co-trimoxazol (9%), and gentamicin (4%). The four gentamicin-resistant isolates were investigated in molecular details. Only three (chloramphenicol) resistant, typical plant-associated enterococci were isolated from overnight enrichment cultures. In conclusion, a contribution of sprouts contaminated with multiresistant, Gram-negative enterobacteria to a common gene pool among human commensal and pathogenic bacteria cannot be excluded.

Keywords: Antibiotic-resistance / Enterobacteria / Enterococci / Foodstuff

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1 Introduction

Resistance to antimicrobial agents can arise either from mutations in the bacterial genome or from acquisition of genes encoding for resistances. Resistance spreads vertically *via* clonal dissemination or horizontally between different isolates, species, genera, and up to different kingdoms [1 – 4]. Distribution of resistance genes is facilitated by the presence of resistance genes on transferable elements and a use of antibiotics in a way that allows direct selection or co-selection of multiresistance [5 – 8]. Hospitals and commercial animal husbandry are prime areas of antibiotic resistance development [9 – 11]. Antibiotic-resistant bacteria and their resistance genes can spread beyond the range of selective pressure. In this respect, a report on fecal carriage of antibiotic resistant coliforms by children who had no previous contact with antibiotics is of particular interest [12]. Probably, different routes of dissemination of resistant bacteria or their resistance genes between these two ecosystems exist, the main route is from animals to humans *via* meat products (*e.g.*, spread of tetracycline and streptothricine resistance in *Escherichia coli*, fluorquinolone-resistant *Campylobacter* spp. or vancomycin-resistant *Enterococcus faecium*) [7, 10, 13]. Also in plant agriculture large amounts of antibiotics are used which could lead to a selection of resistant bacteria [14, 15]. Applying manure from animal farming to agricultural fields could also spread antibiotic-resistant bacteria to plants. Plant foodstuffs have often been debated as a vector transferring pathogenic and/or resistant bacteria. Transfer of pathogens could easily been followed in retrospective by isolating the spreading source for bacterial outbreaks (*E. coli* O*157*, *Salmonella*) [16 – 19]. However, a transfer of (resistant) bacteria themselves is one aspect of resistance transfer only: Bacteria serving as a reservoir for resistance determinants with regard to acquisition, maintaining, and dissemination of resistance determinants may have a much greater influence on resistance gene transfer in nature and natural habitats, *e.g.*, the human colon [7, 10, 12]. To investigate this, we screened potentially raw-

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Abbreviations: cfu, colony-forming unit; **MIC**, minimal inhibitory concentration; **MUG**, 4-methylumbelliferyl-β-D-glucuronide

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eaten vegetables (lettuce, tomato, sprouts, *etc.*) and some fruits (strawberry; Table 1) for a contamination with bacteria having reservoir function, such as coliform bacteria (*Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Escherichia* spp., *Hafnia* spp., *Serratia* spp.) and enterococci.

2 Materials and methods

2.1 Origin of samples, sample preparation, selective media, and supplements

Altogether 20 vegetables of different kinds, but preferably eaten uncooked, were bought in regular supermarkets and at marketplaces in and around Wernigerode/Saxony-Anhalt. If possible, we tried to get samples packed in plastic foils to avoid hand contamination. The 20 samples included: carrots ($n = 1$), cauliflower (1), mushrooms (2), lamb's lettuce (2), strawberry (1), chicory (1), iceberg lettuce (1), rocket salad (1), lettuce mix (1) and tomato (2), mung bean sprouts (3), beluga lens sprouts (1), lens sprouts (1), fenugreek sprouts (1), and black lens sprouts (1). All samples were treated as follows: the samples were divided into three parts: (i) 25 g added to 225 mL NaCl-peptone bouillon (0.85% NaCl, 0.1% casein peptone; pH 7.0), (ii) 25 g added to 225 mL laurylsulfate bouillon (Oxoid, Hampshire, UK), and (iii) 25 g added to 225 mL Enterococcosel bouillon (Becton Dickinson, Sparks, MD, USA). All three prepared samples were homogenized using a MIX 1 apparatus from AES Laboratoire (Combourg, France). Dilutions from sample 1 were directly streaked onto Urine 3 agar II plates (U3G) supplemented with ampicillin (10 mg/L), kanamycin (50 mg/L), streptomycin (50 mg/L), gentamicin (10 mg/L), tetracycline (5 mg/L), or chloramphenicol (10 mg/L; antibiotic-free plates as control) for a semiquantitative analysis of cell counts. U3G was self-prepared according to the instructions of the manufacturer (Heipha, Eppelheim, Germany) and controlled with reference strains. U3G contains peptones, phosphate buffer, and sodium deoxycholate, adjusted to pH 6.8 – 7.0. A mixture of chromogenic/fluorogenic substrates was added aseptically, subsequently poured in Petri dishes. Cell numbers are given in colony-forming units (cfu) per g vegetable. Samples 2 and 3 were incubated overnight at 37°C, appropriate dilutions were streaked onto U3G plates (see above) and Enterococcosel agar plates, respectively, the latter supplemented with vancomycin (50 mg/L), chloramphenicol (10 mg/L), streptomycin (200 mg/L), and gentamicin (100 mg/L; antibiotic-free plates as control). Isolates on U3G agar were preselected due to their color and colony morphology. Color detection depended on the presence or absence of two enzymes, β-galactosidase (substrate Salmon™ Gal; Biosynth AG, Staad, Switzerland) and β-glucosidase (substrate X-Glu; Biosynth AG) [20]. *E. coli* was further identified by fluorescing colonies after excitation with UV light on U3G agar supplemented with MUG (4-methylumbelliferyl-β-D-glucuronide; Biosynth AG) utilizing β-D-glucuronidase. Only colored colonies were further tested for coliform bacteria (white or transparent colonies were partly tested and identified as *P. aeruginosa*, coagulase-negative staphylococci or fungi which confirmed the preselection scheme by color detection). Preselected species were confirmed by Gram stain, selective tests (oxidase/katalase/L-proline aminopeptidase; [21]) and colony morphology/color on bile-chrysoidin-glycerol agar (GCG, SIFIN, Berlin, Germany) and blood agar (*e.g.*, hemolysis). A number of enzyme and sugar fermentation tests including acids from glucose, lactose, and malonate utilization, tryptophan deaminase, lysine-ornithine decarboxylase, and motility tests in semisolid agar were used for further characterizations [21a]. If results were ambiguous, a set of 47 reactions according to Farmer *et al.* [21a], was done. In the case of still contrary results, species-specific PCRs or sequencing of 16S rDNS specific DNA fragments were performed. Mung bean sample 1 was washed similar as to procedures in private households; it was placed into a sterilized plastic bag, sterilized and distilled tap water was added and all was shaked manually for 20 – 30 s. Five samples of wholefood vegetable (fennel, lamb's lettuce, red leaf salad, green leaf salad, carrots), bought at a wholefood market in Brunswick/Lower Saxony, were chosen for comparison. Samples were treated as described above for "common vegetables" and sprouts. Seeds of sprouts were raised in sterile water 48 h at room temperature and treated as described (see above).

2.2 Antibiotic susceptibility testing and antibacterial testing

All strains were tested for antibiotic susceptibilities by microbroth dilution according to the standards of DIN (German Institute for Standards). Test schemes for *E. coli/Salmonella* spp. were taken for gram-negative bacteria. MIC breakpoints are given in µg/mL and are as follows (*s*, susceptible, *r*, resistant): ampicillin, cefotaxim ($s \leq 2$, $r \geq 16$); mezlocillin, mezlocillin/sulbactam, ceftazidim, kanamycin, amikacin, co-trimoxazol ($s \leq 4$, $r \geq 32$); cefotiam, gentamicin ($s \leq 1$, $r \geq 8$); cefoxitin ($s \leq 1$, $r \geq 2$), streptomycin ($s \leq 8$, $r \geq 32$), nalidixic acid ($s \leq 16$, $r \geq 32$), chloramphenicol ($s \leq 8$, $r \geq 16$), tetracycline ($s \leq 1$, $r \geq 8$), and ciprofloxacin ($s \leq 1$, $r \geq 4$). MICs for enterococci were: penicillin, ampicillin ($s \leq 8$, $r \geq 16$), gentamicin (high-level $r \geq 1024$), streptomycin (high-level $r \geq 2048$), erythromycin, clindamycin, tetracycline ($s \leq 1$, $r \geq 8$), chlormaphenicol ($s \leq 8$, $r \geq 16$), vancomycin, teicoplanin ($s \leq 4$, $r \geq 16$), ciprofloxacin, moxifloxacin ($s \leq 0.25$, $r \geq 2$), co-trimoxazol ($s \leq 4$, $r \geq 32$), rifampicin ($s \leq 0.5$, $r \geq 1$), fusidic acid ($s \leq 2$, $r \geq 4$), quinupristin/dalfopristin ($s \leq 1$, $r \geq 4$), and linezolid

($s \leq 4$, $r \geq 8$). All "common vegetables" homogenized in NaCl peptone bouillon were tested for antibacterial activity in agar diffusion tests. Holes of 1 cm diameter punched in the agar were filled with the processed fluids. The plates were overlaid with the sensitive indicator strain *Bacillus subtilis* ATCC 6633. Inhibition zones around the holes would indicate antibacterial activity.

2.3 Genetical and molecular experiments

Transformation and conjugation were performed using standard methods [22]. *E. coli* IHE CV601 served as recipient for conjugations [23]. For transfomation experiments *E. coli* XL1 Blue MR (Δ(*mrc*A)183 Δ(*mcr*CB-*hsd*SMR-*mrr*)173 *end*A1 *sup*E44 *thi*-1*rec*A1 *gyr*A96 (nalidixic acid resistance) *rel*A1 *lac*; Stratagene, Amsterdam, Netherlands) was used as a recipient. PCR was done in a final volume of 25 µL using 10–50 ng genomic DNA, 100 µM primers, and Ready-to-go beads (Amersham Biosciences, Piscataway, NJ, USA). The four gentamicin-resistant isolates were checked for possession of one of the most common gentamicin resistance determinants in coliform bacteria, *ant*(2)-Ia, *aac*(3)-IIc, and *aac*(3)-IVa. Primers were ant(2)-Ia-F: 5′-GGGCGCGTCATGGAGGAGTT and ant(2)-Ia-R 5′-TATCGCGACCAAAGCGGC, aac(3)-IIc-F: 5′-TGAAACGCTGACGGAGCCTC and aac(3)-IIc-R: 5′-GTCGAACAGGTAGCACTGAG, aac(3)-IVa-F: 5′-GTGTGCTGCTGGTCCACAGC and aac(3)-IVa-R: 5′-AGTTGACCCAGGGCTGTCGC [24–26]. Amplification was performed at annealing temperatures of 62, 58, and 60°C for the corresponding fragments of *ant*(2)-Ia, *aac*(3)-IIc, and *aac*(3)-IVa, respectively. PCRs for the two most common chloramphenicol acetyltransferase genes in enterococci, cat_{pIP501} and cat_{pC194} were performed with the following primers cat-pIP501-F 5′-GGATATGAAATTTATCCCTC and cat-pIP501-R: 5′-CAATCATCTACCCTATGAAT, cat-pC194-F: 5′-ATAACCTAACTCTCCGTCGC and cat-pC194-R: 5′- GATTTAGACAATTGGAAGAG [27–29]. Amplification was done at an annealing temperature of 50°C. Labeling was done by incorporating digoxigenin-labeled dUTP into the DNA copy during PCR. Labeling, Southern blot and hybridization were done according to the manufacturer's recommendations (Roche Biochemicals, Mannheim, Germany).

3 Results

3.1 Selective media

The chromogenic U3G agar contains the substrates Salmon-Gal, X-Glu, and MUG allowing a direct color and fluorescence detection and differentiation of various enterobacteria. The system was evaluated with well-characterized tests strains (*Enterobacteriaceae*, *Pseudomonas aeruginosa*, and Gram-positive bacteria as *Enterococcus* spp., *Staphylococcus* spp.) and was successfully applied to our study design. Because not all representatives of a species possess the corresponding enzymes [21] also noncolored colonies were further tested. All of these were identified as *Pseudomonas* spp. or fungi indicating high specificity of our test system.

3.2 Bacterial counts on vegetables

Bacterial counts were calculated from plating dilutions of processed vegetable samples treated as described (see Section 2). Corresponding data for coliform bacteria are given in Table 1. It seemed reasonable to distinguish between "common vegetables" and sprouts. Sprouts showed an about 10^2–10^7 higher contamination in the overall bacterial counts including coliform bacteria (10^7–10^9 colony forming units per g vegetable, cfu/g) when compared to lettuce, tomato, *etc.* (10^2–10^5 cfu/g) which were grouped together as "common vegetables". Exceptions among the latter group were mushrooms and already processed samples such as "lettuce mix" with numbers of up to 10^7 cfu/g of (coliform) bacteria. In some samples of this group about 10^5 to 10^7 cfu/g but no coliform bacteria were identified (*e.g.*, lamb's lettuce 2 and iceberg lettuce 1/2). This was in contrast to the sprout samples where all identified bacteria belonged to the coliform flora. Washing did not result in a reduction of the overall bacterial counts; cfu of coliform bacteria per g mung bean sprout sample 1 dropped after a washing step only threefold from 1.2×10^8 to 3.9×10^7 (not shown in detail).

When we compared antibiotic-resistant coliform bacteria in relation to the overall coliform flora, we found some orders of magnitude lower bacterial counts in "common vegetable" samples than in sprouts. Numbers varied according to the kind of vegetable and antibiotic. Highest numbers were found for ampicillin which included also isolates with intrinsic ampicillin resistance, followed by resistances to tetratcycline, kanamycin, and chloramphenicol (Table 1). Resistances to streptomycin and gentamicin were rare. Numbers for sprouts were completely different. All samples were characterized by high numbers of antibiotic-resistant coliformes including bacteria resistant to ampicillin (about 10^7 cfu/g including isolates with intrinsic ampicillin resistance), tetracycline (about 10^4 cfu/g), streptomycin (10^3–10^6 cfu/g), chloramphenicol (0–10^5 cfu/g), kanamycin (0–10^7 cfu/g), and gentamicin (0–10^2 cfu/g).

Growth inhibition by vegetable secondary metabolites was tested using a modified agar diffusion test. No inhibition zones around the holes in the agar supplemented with vegetable extracts were detected indicating no antibacterial activity of the raw or processed "common vegetables".

Table 1. Overall bacterial counts (cfu/g) and number of antibiotic-resistant bacteria grown on selective plates

Vegetable	a) All bacteria b) Coliform bacteria	Numbers of bacteria grown on selective plates containing[b)]					
		AMP	KAN	GEN	STR	CMP	TET
Carrots, *Daucus carota*	a) 5.6×10^4 b) 3.1×10^4	a) 2.1×10^4 b) 10^3	a) 2×10^2 b) ∅	∅	a) 2.1×10^3 b) ∅	a) 10^4 b) ∅	a) 1.6×10^3 b) 10^2
Cauliflower, *Brassica oleraceae*	a) 9×10^2 b) 9×10^2	a) 5×10^2 b) 5×10^2	∅	∅	∅	∅	∅
Mushrooms 1, *Agaricus hortensis*	a) 1.4×10^4 b) 1.4×10^4	a) 2.2×10^3 b) 7×10^2	a) 10^2 b) ∅	∅	a) 3×10^3 b) ∅	∅	a) 10^2 b)∅
Mushrooms 2, *A. hortensis*	a) 1.3×10^7 b) 1.3×10^7	a) 7.4×10^6 b) 2.6×10^3	a) 9.7×10^6 b) 3.4×10^4	a) 1.8×10^6 b) ∅	a) 1.7×10^6 b) ∅	a) 1.6×10^6 b) ∅	a) 1.7×10^6 b) ∅
Strawberry, *Fragaria* spp.	a) 2.3×10^5 b) 2.3×10^5	∅	∅	∅	∅	∅	∅
Lamb's lettuce 1, *Valerianella locusta*	a) 3.4×10^5 b) 3.4×10^5	a) 6×10^4 b) 6×10^4	a) 4.7×10^3 b) 4.7×10^3	∅	∅	a) 10^5 b) ∅	∅
Lamb's lettuce 2, *V. locusta*	a) 1.1×10^7 b) ∅	a) 4.9×10^7 b) ∅	a) 5.9×10^4 b) ∅	a) 1.3×10^5 b) ∅	a) 3.5×10^6 b) ∅	a) 5.4×10^6 b) ∅	∅
Iceberg lettuce 1, *Lactuca sativa*	a) 4.8×10^6 b) ∅	a) 3.6×10^6 b) ∅	a) 10^6 b) ∅	a) 4.8×10^6 b) ∅	a) 4.7×10^4 b) ∅	a) 2.4×10^4 b) ∅	∅
Iceberg lettuce 2, *L. sativa*	a) 6.6×10^5 b) ∅	a) 1.1×10^6 b) ∅	∅	∅	∅	a) 3.8×10^3 b) ∅	∅
Tomato 1, *Solanum lycopersicum*	a) 8.8×10^4 b) 3.1×10^4	a) 6.3×10^4 b) 3.7×10^4	a) 1.9×10^4 b) 1.9×10^4	a) 1.8×10^4 b) ∅	a) 3.9×10^4 b) ∅	a) 2.2×10^4 b) ∅	a) 2×10^4 b) 1.5×10^3
Tomato 2, *S. lycopersicum*	∅	∅	∅	∅	a) 1.5×10^2 b) ∅	a) 7×10^2 b) ∅	a) 5×10^1 b) ∅
Rocket salad, *Rucola selvatica*	a) 2.5×10^7 b) 1.4×10^7	a) 6.8×10^6 b) 7.2×10^5	a) 2.8×10^5 b) 7×10^2	a) 3.4×10^5 b) ∅	a) 4.7×10^5 b)	a) 10^6 b) ∅	a) 1.1×10^4 b) 3×10
Mixed salad	a) 5.2×10^7 b) 2.3×10^7	a) 5.6×10^6 b) 5.6×10^6	a) 1.6×10^5 b) 10^5	∅	a) 2.6×10^3 b) 1.5×10^3	a) 2.7×10^3 b) 3.5×10^2	a) 1.8×10^4 b) 1.5×10^4
Chicory, *Cichorium intybus var. foliosum*	a) 8.5×10^5 b) 8.5×10^5	a) 3.2×10^5 b) 3.2×10^5	∅	∅	a) 5×10^1 b) 5×10^1	a) 3.5×10^1 b) 3.5×10^1	∅
Mung bean sprouts 1 *Phaseolus aureus*	a) 1.2×10^8 b) 1.2×10^8	a) 7×10^7 b) 7×10^7	a) 1.6×10^7 b) 1.6×10^7	a) 2.3×10^4 b)	a) 2.2×10^4 b) 2.2×10^4	a) 2.8×10^4 b) 2.8×10^4	a) 3.5×10^4 b) 3.5×10^4
Mung bean sprouts 2, *P. aureus*	a) 5.9×10^8 b) 5.9×10^8	a) 1.5×10^8 b) 1.5×10^8	a) 6.2×10^5 b) 6.2×10^5	a) 1.1×10^5 b) ∅	a) 3.3×10^6 b) 3.3×10^6	a) 9×10^6 b) ∅	a) 5.2×10^5 b) 5.2×10^5
Mung bean sprouts 3[a)] *P. aureus*	a) 2.6×10^8 b) 2.6×10^8	a) 6.7×10^7 b) 6.7×10^7	a) 1×10^5 b) 5×10^2	a) 4×10^2 b) ∅	a) 3.4×10^4 b) 3.4×10^4	a) 1.2×10^5 b) 1.2×10^5	a) 2.5×10^4 b) 2.5×10^4
Beluga lens sprouts[a)], *Lens culinaris* spp.	a) 1.1×10^8 b) 1.1×10^8	a) 1.3×10^7 b) 1.3×10^7	a) 2.6×10^6 b) 2.6×10^6	a) 1×10^2 b) 1×10^2	a) 3×10^3 b) 3×10^3	a) 4×10^4 b) ∅	a) 3.4×10^3 b) 3.4×10^3
Black lens sprouts[a)] *L. cul. Nigrans*	a) 2.5×10^8 b) 2.5×10^8	a) 7.1×10^6 b) 2.8×10^6	a) 3.5×10^4 b) ∅	∅	a) 2.2×10^4 b) 2.1×10^4	a) 3.4×10^4 b) ∅	a) 2.4×10^4 b) 2.4×10^4
Fenugreek[a)] *Trigonella foenum graecum*	a) 2.4×10^9 b) 2.4×10^9	a) 2.1×10^8 b) 1×10^8	a) 5.1×10^7 b) 3×10^2	a) 1.7×10^4 b) ∅	a) 1.3×10^6 b) 1.3×10^6	a) 2.6×10^6 b) 3×10^4	a) 6.2×10^5 b) 6.2×10^5

cfu/g, colony-forming units per g raw vegetable; ∅, no bacteria found; several samples of the same kind were all from different producers.
a) Identical manufacturing company in Saxony
b) AMP, ampicillin; KAN, kanamycin; GEN, gentamicin; STR, streptomycin; CMP, chloramphenicol; TET, tetracycline

It was to suppose that resistant bacteria are already present on seeds from which sprouts have been grown. To investigate this, we performed experiments with five different samples (seeds of chickpeas, black lens, brown lens, fenugreek, and mung beans) simulating swelling reactions as in the manufacturer's farms but with sterilized tap water instead of normal tap water. We found bacteria only in three samples ($10^6 – 10^8$ cfu/g), however, single coliform bacteria

were only detected on antibiotic-free plates and on selective plates supplemented with ampicillin (not quantified). There was no bacterial growth on agar plates containing antibiotics other than ampicillin. All coliform bacteria appeared dark blue on U3G agar were identified as *Pantoea agglomerans* (formerly *Enterobacter a.*) possessing an intrinsic ampicillin resistance.

3.3 Identification of bacterial species and resistance patterns

Colonies on U3G agar enriched with different supplements allowed a preselection due to different colony colors. Not all representatives of coliform bacteria possess enzymes converting the given supplements into a colored precipitate (*e.g.*, *Citrobacter freundii* converts Salmon-Gal only in 87%, respectively [21]). According to that also noncolored isolates were partly characterized. None of these were members of *Enterobacteriaceae*. The majority emerged as oxidase-positive *Pseudomonas* ssp. or fungi.

Altogether 92 isolates were further characterized by the methods described above (41 from "common vegetable", 51 from sprouts). Most common genera and species were: *Enterobacter* spp. (n = 25, including 19 *E. cloacae*), *Citrobacter* spp. (n = 22, including 8 *C. freundii*), *Klebsiella* spp. (n = 21, including 9 *K. pneumoniae*), *Serratia* spp. (n = 9), *Escherichia coli* (n = 8), and *Pantoea agglomerans* (n = 5). Among all 92 coliform bacteria resistances were distributed as follows: 43% against tetracycline, 29% against chloramphenicol, 26% against kanamycin, 37% against streptomycin, and 4% against gentamicin. Forty-seven % were resistant against sulfamerazin and 9% against co-trimoxazol. Resistances to these antibiotics have not been directly selected indicating a wide distribution of these determinants among the identified coliform bacteria.

In general, isolates from "common vegetables" could be differentiated from isolates from sprouts due to their resistance phenotypes. Bacteria isolated from direct cultures (no pre-enrichment steps) originating from "common vegetables" possessed in the majority only single or double resistances whereas those from sprouts were characterized by three to five resistances. The majority of isolates (n = 7/11) with resistances to more than five antibiotics were identified only after pre-enrichment, these multiresistant coliform bacteria are given in Table 2.

The intrinsic AmpC β-lactamase of various *Enterobacteriaceae* resulted in a large number of strains appearing ampicillin-resistant; due to that, percentages for ampicillin resistance were not provided. However, some interesting resistance features could be demonstrated for single strains. *Citrobacter freundii* 17.1 was resistant against mezlocillin and different cephalosporines (cefoxitin, cefotiam) including newer ones like cefotaxime and ceftazidime (Table 2) suggesting presence of an overexpressed AmpC β-lactamase. An isolate which could not be further specified (identified as "enteric group 59") was besides ampicillin and cefoxitin also resistant against cefotiam (second generation cephalosporine). *Serratia* spp. 26.6 also possessed a cefotiam resistance (AMP, CTM, CMP, TET, SMZ; for legend see Table 2). A single *E. coli* isolate 16.27 showed resistance to ciprofloxacin, a second generation fluorquinolone.

Altogether, three antibiotic-resistant enterococcal isolates were selected from pre-enrichment cultures on selective agar plates. Two *E. pseudoavium* (one from lamb's lettuce 1, the other from lettuce mix) and a single *E. mundtii* (lamb's lettuce 1) were identified which belong to species known to be plant-associated. Isolates appeared borderline resistant to fusidic acid (MIC = 4 μg/mL), rifampin (only

Table 2. Coliform bacteria with more than five antibiotic resistances

Origin	Strain	Genus/species	Resistance phenotype
Tomato (a)	22.18	*Enterobacter cloacae*	AMP(i), COX, NAL, CMP, SMZ, SXT
Chicory (b)	17.1	*Citrobacter freundii*	AMP, MZL, COX, CTM, CTX, CAZ, STR
Rocket salad (b)	25.47	*Klebsiella pneumoniae*	AMP, STR, TET, CMP, SMZ, SXT
Lettuce mix (a)	26.40	Enteric group 59/*Buttiauxella noackie*	AMP, COX, CTM, NAL, CMP, TET, SMZ
Mung bean	15.5	*Escherichia coli*	AMP, MZL, STR, TET, SMZ, SXT
sprouts 1 (b)	15.22	*Escherichia coli*	AMP, MZL, GEN, STR, TET, NAL, SMZ, SXT
	15.33	*Klebsiella pneumoniae*	AMP, GEN, STR, TET, CMP, SMZ, SXT
	16.27	*Escherichia coli*	AMP, MZL, GEN, KAN, STR, TET, NAL, CIP, CMP, SMZ, SXT
	16.33	*Klebsiella pneumoniae*	AMP, STR, KAN, TET, SMZ, SXT
Beluga lens	28.2	*Pantoea agglomerans*	AMP, MZL, GEN, KAN, STR, TET, CMP, SMZ
Sprouts (a)	28.5	*Serratia* spp.	AMP(i), COX, STR, CMP, TET, SMZ

AMP/(i), ampicillin/(intermediately resistant); CAZ, ceftazidime; CIP, ciprofloxacin; CMP, chloramphenicol; COX, cefoxitin; CTM, cefotiam; CTX, cefotaxime, GEN, gentamicin; KAN, kanamycin; MZL, mezlocillin; NAL, nalidixic acid; TET, tetracycline; SMZ, sulfamerazin; STR, streptomycin; SXT, co-trimoxazol. Gentamicin resistance is underlined.

a) From original sample

b) From pre-enrichment culture

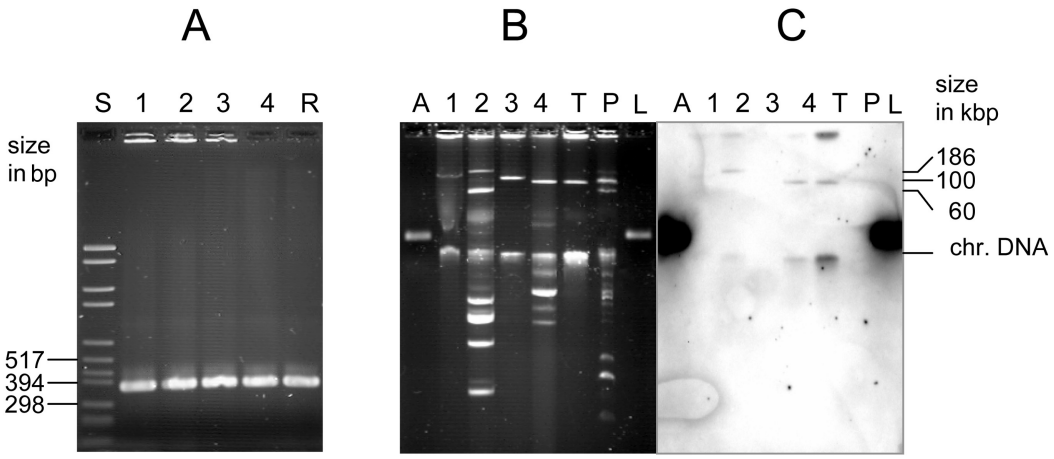

Figure 1. Gentamicin resistance genes in coliform bacteria from sprouts. (A) PCR amplicon of the *aac*(3)-IIc gene. (B) Plasmid profiles of gentamicin-resistant Gram-negative bacteria. (C) Corresponding Southern blot hybridized with a labeled *aac*(3)-IIc gene probe. Legend: 1, *K. pneumoniae* 15.33; 2, *P. agglomerans* 28.2; 3, *E. coli* 16.27; *E. coli* 15.22; P, PCR product of the *aac*(3)-IIc gene; S, size marker; R, *E. coli* reference isolate for *aac*(3)-IIc gene; A, PCR product of the *aac*(3)-IIc gene; L, labeled PCR product of the *aac*(3)-IIc gene; T, transformant of *E. coli* 15.22 × XL1; P, plasmid size markers.

two *E. pseudoavium*; MIC = 1 – 2 µg/mL) and chloramphenicol (only two *E. pseudoavium*; MIC = 16 µg/mL).

3.4 Samples from wholefood vegetable

Altogether five samples of wholefood vegetables were investigated similar as described for "common vegetables" and sprouts (results not given in details). Contamination either with bacteria at all or with coliform bacteria ($3 \times 10^3 – 9.5 \times 10^4$ cfu/g) was less than seen in most vegetables from the former two groups. All but one agar plates supplemented with antibiotics including plates with ampicillin were free of coliform bacteria (except red leaf salad with 10^2 cfu/g on plates with ampicillin). These results indicate a some orders of magnitude lower contamination of wholefood vegetables with both coliform bacteria and antibiotic-resistant coliform bacteria compared to commonly grown vegetables.

3.5 Molecular tests

PCRs for the two most common chloramphenicol acetyltransferase genes in enterococci, cat_{pIP501} and cat_{pC194}, were negative for both chloramphenicol-resistant *E. pseudoavium* isolates. Totally, four Gram-negative isolates were gentamicin-resistant (Table 2) including *K. pneumoniae* 15.33, *P. agglomerans* 28.2, and two *E. coli* 15.22 and 16.27. PCR screenings for the three most common gentamicin resistance genes among coliform bacteria (*ant*(2)-Ia, *aac*(3)-IIc and *aac*(3)-IVa) revealed a fragment specific for *aac*(3)-IIc (Fig. 1A). Southern blot hybridization localized the corresponding resistance gene on different sized plasmids (80 – 120 kb) in the two *E. coli* and the single *K. pneumoniae* isolates (Fig. 1 B/C). The resistance determinant could not be conjugatively transferred from one of the donors into an *E. coli* laboratory recipient CV601. An *E. coli* recipient XL1 could successfully be transformed only with the resistance plasmid from *E. coli* 15.22 (Fig. 1 B/C).

4 Discussion

4.1 Bacterial counts

We investigated 20 vegetables of different kinds and from different producers for antibiotic resistant enterobacteria. Based on our results we divided them into two groups: sprouts and "non-sprouts" designated as "common vegetables". "Common vegetables" were some orders of magnitude lesser contaminated with coliform bacteria than sprouts (Table 1). An exception were "common vegetable" samples which were processed before packaging such as rocket salad and lettuce mix showing bacterial counts higher than nonprocessed "common vegetables" and somehow lower than sprouts. Intact bacterial biofilms on vegetables prevent colonization with fecal or facultative pathogenic bacteria [30]. During processing (trimming, slicing, washing, dehydrating, *etc.*) such biofilms are destroyed, excreted plant extracts serve as nutritional sources for a subsequent colonization with other (human/fecal ?) bacteria. Some enterococcal species (*E. faecium, E. faecalis*) and *E. coli* are classified as fecal indicator bacteria [31, 32], other coliformes such as *Klebsiella* spp., *Enterobacter* spp.,

and *Citrobacter* spp. could besides this be recognized as bacteria with environmental and plant reservoirs [33]. Consequentially, we did not identify fecal indicator bacteria in nonprocessed "common vegetables", but in processed vegetables (rocket salad – *E. coli*) and in sprouts (*E. coli*). Grimont *et al.* [34] showed that *K. pneumoniae* from humans did not convert 5-keto-gluconate, whereas environmental strains do so. All nine but one isolates were 5-keto-gluconate-negative. Of these eight isolates six were from sprouts and two were from rocket salad suggesting a human contamination most probably during processing [35], the single 5-keto-gluconate-positive isolate was from mushrooms.

Differences in cell counts between "common vegetables" and sprouts did not result from antibacterial substances released from the former. Reference isolate *B. subtilis* ATCC 6633 was not inhibited when tested against vegetable extracts in agar diffusion assays.

Washing "mung bean sprout sample 1" with sterilized, distilled water did not alter bacterial counts significantly (decrease from 1.2×10^8 to 3.9×10^7 cfu/g). As has been described recently, bacteria on sprouts reside in biofilms which cannot be removed by simple washing [36, 37].

4.2 Antibiotic resistances

Samples from sprouts showed more colonies on antibiotic-containing agar plates when compared to "common vegetables" (Table 1) and these bacteria appeared to be more frequently multiresistant than isolates from "common vegetables" (Table 2). More than three different types of resistance were very rare among "common vegetable" isolates, whereas three to four resistances were the average numbers for sprout isolates. According to Table 2 isolates with more than five resistance characters were in 7/11 cases from sprouts including all four identified gentamicin-resistant strains. Among 11 multiresistant coliformes seven were identified from pre-enrichment cultures suggesting their quantitative inferiority among the overall bacterial flora of that vegetables. Nevertheless, pre-enrichments were performed none-selectively, the cultures were free of antibiotics.

Only a few authors described antibiotic-resistant commensal bacteria contaminating vegetable foodstuffs [38 – 40]. In none of these studies antibiotic-resistant bacteria have been directly selected as performed here. Due to this fact, data resulting from our study cannot be compared to the results from the others without any bias. Oesterblad and co-workers identified among 137 vegetable samples a total of 535 strains [39]. Among these, *Enterobacter* spp. were most frequently (n = 150; 28%) whereas *E. coli* was rare (n = 22; 4%), two findings which were also confirmed in our study. Second most common was *Enterobacter agglomerans* (n = 117; 22%) which is now classified as *Pantoea agglomerans*. Distributions of other genera were: 73 *Klebsiella* spp. (14% including 35 *K. pneumoniae*; 7%), 52 *Citrobacter* spp. (10%) and 48 *Serratia* spp. (9%). Due to the different selection strategies prevalences of antibiotic resistances were much lower than that found in our study; 12% chloramphenicol, 6% tetracycline, and <1% trimethoprim resistance. None of the 535 strains was resistant against cefotaxime, nalidixic acid, ciprofloxacin, or gentamicin. In contrast, we could identify: (i) six nalidixic acid resistant isolates (*E. cloacae* 22.18, "enteric group 59" 26.40, *E. coli* 25.40, *Citrobacter* spp. 26.29, *E. coli* 15.22, *E. coli* 16.27) including a single *E. coli* 16.27 which was also resistant against ciprofloxacin, and (ii) a single *C. freundii* 17.1 which was multiresistant against β-lactams including second-generation cephalosporines, such as cefotaxime and ceftazidime (Table 2). Also seven mezlocillin-resistant isolates including four *E. coli* (*E. coli* 25.40, *C. freundii* 17.1, *E. coli* 15.5, *E. coli* 15.22, *E. coli* 16.27, *K. ozeanae* 16.31, *P. agglomerans* 28.2) were identified, but these figures could not be compared since mezlocillin was not tested in the former study [39].

Stock and co-workers [41] investigated antibiotic susceptibilities among the *Serratia marcescens* and *S. liquefaciens* complex. *S. marcescens* and *S. liquefaciens* were identified to be intrinsic intermediately resistant and intrinsically resistant to tetracyclines, respectively. Of our nine *Serratia* isolates, a single *S. marcescens* and two *S. rubidea* were identified. Definite species could not be assigned to the other six isolates by our test schemes. All nine *Serratia* were tetracycline-resistant. We are aware of the fact, that our overall prevalences for tetracycline resistance could be biased by a natural resistance in some *Serratia* species.

High numbers of *Enterobacteriaceae* on sprouts did not result simply from a contamination of sprout seeds. We tested five different seed samples originating from the same manufacturer providing four sprout samples (Table 1, last four samples). The only species detectable was *P. agglomerans* (no antibiotic resistances). This was in striking discrepancy to (antibiotic-resistant) bacteria identified in the corresponding sprout samples (Tables 1 and 2; black lens, mung bean (sample 3) and fenugreek seeds were tested) suggesting a contamination with the identified enterobacteria during food handling and processing or *via* tap water.

4.3 Molecular characterizations

Altogether four different gentamicin-resistant coliformes were identified (Table 2). Gentamicin is not used in plant agriculture, a contamination *via* human or animal sources was probable. However, *P. agglomerans* (28.2, gentamicin-resistant; Table 2) is expected to be rather environmentally

distributed or plant-associated than a fecal colonizer. At least for this case a horizontal gene transfer could be supposed. Mating and transformation experiments revealed no conjugatively transferable plasmid/element and only one plasmid from *E. coli* 15.22 which could be transformed into and maintained in *E. coli* host XL1 Blue MR. Resistance was in all cases mediated *via* the wide-spread aminoglycoside acetyltransferase *aac*(3)-IIc [42 – 44]. Summarized results for gentamicin resistance are ambiguous, a common gene pool including bacteria from sprouts could neither be ruled out nor supported.

4.4 Enterococci from vegetables

A number of reports described distinct enterococcal species associated with plants [45, 46], other species known to be facultative human pathogens appeared as contaminants on vegetables [47]. Some already known enterococcal species were confirmed as colonizers of the epiphytic microflora of grass (*E. sulfureus*, *E. casseliflavus*, *E. mundtii*) and a completely new species was described based on different methods [45]. The facultative pathogenic species *E. faecalis* and *E. faecium* were rarely found (7.9% and 5.2%; [45]). In a report from Argentina, a variety of enterococci was identified on 70 lettuce samples including *E. faecalis* and *E. faecium* (32.6% and 21.7%; [47]). High-level resistance against streptomycin was found in six isolates (4 *E. faecium*, 1 *E. hirae*, 1 *E. mundtii*) including two *E. faecium* which were also gentamicin-resistant.

Our results on enterococci were rather surprising. Numbers of enterococci at all were negligible when compared to the numbers of all bacteria or all coliformes (not shown in detail; Table 1). All three antibiotic-resistant enterococci identified were from pre-enrichment cultures and were selected on plates supplemented with chloramphenicol. None of the two most common chloramphenicol acetyltransferase genes in enterococci [27, 28, 48] were found by PCR. Due to our results enterococci on vegetables including sprouts do not contribute to a common resistance gene pool among *Enterococcus*. Studies on the facultative pathogenic enterococci, *E. faecium* and *E. faecalis*, from human stool samples with or without previous hospital contact revealed much higher resistance rates as found here [49, 50].

4.5 Conclusions

Our study revealed different results for the two groups of vegetables, "common vegetables" and sprouts. "Common vegetables" were some orders of magnitude lower contaminated with coliform bacteria and possessed only few antibiotic-resistant bacteria whereas sprouts were highly contaminated ($\geq 10^8$ cfu/g) with coliform bacteria including many (multi-)resistant isolates. Consumption of seed sprouts has been growing in global popularity as "alternative products" over the past 20 years overall Europe [17]. Outbreaks with pathogenic bacteria spread *via* contaminated vegetables, mainly sprouts, demonstrated how successful bacteria could disseminate *via* this route to humans [17]. Appearance of fluorquinolone and second generation cephalosporine resistance in bacteria identified in our study suggested an animal or human origin since antibiotics of these classes are not used in plant agriculture [14].

In summary, we did not find evidence that coliform bacteria from "common vegetables" contribute substantially to high levels of resistance among bacteria of the human fecal flora. Wholefood vegetables showed an even lesser contamination with both coliform bacteria and antibiotic-resistant coliform bacteria than "common vegetables". This is obviously different from sprouts. Consumption of sprouts could contribute to further dissemination of antibiotic-resistant bacteria and of resistance genes as has already been reported for meat products. It remains to be discussed whether the comparably high bacterial contamination with enterics which are far above the values accepted for drinking water in countries of the European Union needs attention of regulatory bodies. Based on our results a contribution of sprouts to a common gene pool for antibiotic resistances cannot be excluded.

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5 References

[1] Mazodier, P., Davies, J., Gene transfer between distantly related bacteria. *Ann. Rev. Genet.* 1991, *25*, 147 – 171.

[2] Davison, J., Genetic exchange between bacteria in the environment. *Plasmid* 1999, *42*, 73 – 91.

[3] De la Cruz, F., Davies, J., Horizontal gene transfer and the origin of species: lessons from bacteria. *Trends Microbiol.* 2000, *8*, 128 – 133.

[4] Nelson, K. E., Clayton, R. E., Gill, S. R., Gwinn, M. L., *et al.*, Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*. *Nature* 1999, *399*, 323 – 329.

[5] Recchia, G. D., Hall, R.M., Origins of mobile gene cassettes found in integrons. *Trends Microbiol.* 1997, *5*, 389 – 394.

[6] Salyers, A. A., Amabile-Cuevas, C. F., Why are resistance genes so resistant to elimination? *Antimicrob. Agents Chemother.* 1997, *41*, 2321 – 2325.

[7] Witte, W., Medical consequences of antibiotic use in agriculture. *Science* 1998, *279*, 996 – 997.

[8] Melo Cristino, J., Correlation between consumption of antimicrobials in humans and development of resistance in bacteria. *Int. J. Antimicrob. Agents* 1999, *12*, 199 – 202.

[9] Levy, S. B., The 2000 Garrod Lecture – Factors impacting on the problem of antibiotic resistance. *J. Antimicrob. Chemother.* 2002, *49*, 25 – 30.

[10] Tenover, F. C., Development and spread of bacterial resistance to antimicrobial agents: An overview. *Clin. Infect. Dis.* 2001, *33 (Suppl. 3)*, S108 – 115.

[11] Teuber, M., Veterinary use and antibiotic resistance. *Curr. Opin. Microbiol*. 2001, *4*, 493 – 499.

[12] Millar, M. R., Walsh, T. R., Linton, C. J., Zhang, S., *et al.*, Carriage of antibiotic-resistant bacteria by healthy children. *J. Antimicrob. Chemother.* 2001, *47*, 605 – 610.

[13] Levy, S. B., FitzGerald, G. B., Macone, A. B., Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *N. Engl. J. Med.* 1976, *295*, 583 – 588.

[14] Falkiner, F. R., The consequences of antbiotic use in horticulture. *J. Antimicrob. Chemother.* 1998, *41*, 429 – 431.

[15] McManus, P. S., Stockwell, V. O., Sudin, G. W., Jones, A. L., Antibiotic use in plant agriculture. *Ann. Rev. Phytopath.* 2002, *40*, 443 – 465.

[16] Cummings, K., Barrett, E., Mohle-Boetani, J. C., Brooks, J. T., *et al.*, A multistate outbreak of *Salmonella* serotype Baildon associated with domestic raw tomatoes. *Emerg. Infect. Dis.* 2001, *7*, 1046 – 1048.

[17] Taormina, P. J., Beuchat, L. R., Slutsker, L., Infections associated with eating seed sprouts: an international concern. *Emerg. Infect. Dis.* 1999, *5*, 626 – 634.

[18] Long, S. M., Adak, G. K., O'Brien, S. J., Gillespie, I. A., General outbreaks of infectious intestinal disease linked with salad vegetables and fruit, England and Wales, 1992 – 2000. *Commun. Dis. Public Health* 2002, *5*, 101 – 105.

[19] Sagoo, S. K., Little, C. L., Ward, L., Gillespie, I. A., Mitchell, R. T., Microbiological study of ready-to-eat vegetables from retail establishments uncovers a national outbreak of salmonellosis. *J. Food Protect.* 2003, *66*, 403 – 409.

[20] De Siloniz, M. I., Valderrama, M. J., Peinado, J. M., A chromogenic medium for the detection of yeasts with β-galactosidase and β-glucosidase activities from intermediate moisture foods. *J. Food Prot.* 2000, *63*, 651 – 654.

[21] Fanghaenel, S., Reissbrodt, R., Giesecke, H., L-prolineaminopaptidase activity as a tool for identification and differentiation of *Serratia marcescens*, *S. liquefaciens*, *Hafnia alvei* strains. *Zbl. Bakt.* 1991, *275*, 11 – 15.

[21a] Farmer III, J. J., Davis, B. R., Hickman-Brenner, F. W., McWhorter, A., *et al.*, Biochemical identification of new species and biogroups of *Enterobacteriacae* isolated from clinical specimes. *J. Clin. Microbiol*. 1985, *21*, 46 – 76.

[22] Sambrook, J., Fritsch, E., Maniatis, T. (Eds.), *Molecular Cloning – a Laboratory Manual.* Cold Spring Laboratory Press, New York 1989.

[23] Tschaepe, H., Tietze, E., Koch, C., Characterization of conjugative R plasmids representing a new incompatibility group (*Inc*U). *J. Gen. Microbiol.* 1981, *127*, 155 – 160.

[24] Cameron F. H., Groot Obbink, D. J., Ackerman, V. P., Hall, R. M., Nucleotide sequence of the AAD(2″) aminoglycoside adenylyltransferase determined *aadB*. Evolution relationship of this with those surrounding *addA* in R538-1 and *dhfr*II in R388. *Nucleic Acids Res.* 1986, *14*, 8625 – 8635.

[25] Vliegenthart, J. S., Ketelaar van Gaalen, P. A., van de Klundert, J. A., Nucleotide sequence of the aacC2 gene, a gentamicin resistance determinant involved in a hospital epidemic of multiresistant members of the family Enterobacteriacae. *Antimicrob. Agents Chemother.* 1989, *33*, 1153 – 1159.

[26] Brau, B., Pilz, U., Piepersberg, W., Genes for gentamicin-(3)-*N*-acetyltransferases III and IV:I. Nucleotide sequence of an IS*140* element in its expression. *Mol. Gen. Genet.* 1984, *193*, 179 – 187.

[27] Aarestrup, F. M., Agerso, Y., Gerner-Smidt, P., Madsen, M., Jensen, L. B., Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. *Diagn. Microbiol. Infect. Dis.* 2000, *37*, 127 – 137.

[28] Horinouchi, S., Weisblum, B., Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. *J. Bacteriol.* 1982, *150*, 815 – 825.

[29] Werner, G., Hildebrandt, B., Klare, I., Witte, W., Linkage of determinants for streptogramin A, macrolide-lincosamide-streptogramin B, and chloramphenicol resistance on a conjugative plasmid in *Enterococcus faecium* and dissemination of this cluster among streptogramin-resistant enterococci. *Int. J. Med. Microbiol*. 2000, *290*, 543 – 548.

[30] Carmichael, I., Harper, I. S., Coventry, M. J., Taylor, P. W. J., *et al.*, Bacterial colonization and biofilm development on minimally processed vegetables. *J. Appl. Microbiol.* 1999, *85 (Suppl.)*, 45S – 51S.

[31] Pinto, B., Pierotti, R., Canale, G., Reali, D., Characterization of "faecal streptococci" as indictors of faecal pollution and distribution in the environment. *Lett. Appl. Microbiol.* 1999, *29*, 258 – 263.

[32] Fogarty, L. R., Haack, S. K., Wolcott, M. J., Whitman, R. L., Abundance and characteristics of the recreational water quality indicator bacteria *Escherichia coli* and enterococci in gull faeces. *J. Appl. Microbiol.* 2003, *94*, 865 – 878.

[33] Balows, A., Trueper, H. G., Dworkin, M., Harder, W., Schleifer, K.-H. (Eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications. Vol III.* Springer Verlag, New York 1992.

[34] Grimont, F., Grimont, P. A. D., Richard, C., The Genus Klebsiella. In: Balows, A., Trueper, H. G., Dworkin, M., Harder, W., Schleifer, K.-H. (Eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, Vol III.* Springer Verlag, New York 1992, pp. 2775 – 2796.

[35] Kaneko, K., Hayashidani, H., Takahashi, K., Shiraki, Y., *et al.*, Bacterial contamination in the environment of food factories processing ready-to-eat fresh vegetables. *J. Food Prot.* 1999, *62*, 800 – 804.

[36] Fett, W. F., Naturally occurring biofilms on alfalfa and other sprouts. *J. Food Prot.* 2000, *63*, 625 – 632.

[37] Fett, W. F., Cooke, P. H., Scanning electron microscopy of native biofilms on mung bean sprouts. *Can. J. Microbiol.* 2003, *49*, 45 – 50.

[38] Brocklehurst, T. F., Zaman-Wong, C. M., Lund, B. M., A note on the microbiology of retail packs of prepared salad vegetables. *J. Appl. Bacteriol.* 1987, *63*, 409 – 415.

[39] Oesterblad, M., Pensala, O., Peterzéns, M., Heleniusc, H., Huovinen, P., Antimicrobial susceptibility of *Enterobacteriaceae* isolated from vegetables. *J. Antimicrob. Chemother.* 1999, *43*, 503 – 509.

[40] Hamilton-Miller, J. M. T., Shah, S., Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. *Int. J. Antimicrob. Agents* 2001, *18*, 81 – 83.

[41] Stock, I., Grueger, T., Wiedemann, B., Natural antibiotic susceptibility of strains of *Serratia marcescens* and the *S. liquefaciens* complex: *S. liquefaciens sensu stricto, S. proteamaculans*, and *S. grimesii*. *Int. J. Antimicrob. Agents* 2003, *22*, 35 – 47.

[42] Miller, G. H., Sabatelli, F. J., Hare, R. S., Glupczynski, Y., *et al.*, The most frequent aminoglycoside resistance mechanisms-changes with time and geographic area: A reflection of aminoglycoside usage patterns? *Clin. Infect. Dis.* 1997, *24 (Suppl. 1)*, S46 – 62.

[43] Sandvang, D., Aarestrup, F. M., Chracaterization of aminoglycoside resistance genes and class 1 integrons in porcine and bovine gentamicin-resistant *Escherichia coli*. *Microbial Drug Resist.* 2000, *6*, 19 – 27.

[44] Schwarz, S., Chaslus-Dancla, E., Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Vet. Res.* 2001, *32*, 201 – 225.

[45] Mueller, T., Ulrich, A., Ott, E.-M., Mueller, M., Identification of plant-associated enterococci. *J. Appl. Microbiol.* 2001, *91*, 268 – 278.

[46] Ott, E.-M., Mueller, T., Mueller, M., Franz, C. M. A. P., *et al.*, Population dynamics and antagonistic potential of enterococci colonizing the phyllosphere of grasses. *J. Appl. Microbiol.* 2001, *91*, 54 – 66.

[47] Ronconi, M. C., Merino, L. A., Fernandez, G., Detection of *Enterococcus* with high-level aminoglycoside and glycopeptide resistance in *Lactuca sativa* (lettuce). *Enferm. Infecc. Microbiol. Clin.* 2002, *20*, 380 – 383.

[48] Murray, I. A., Shaw, W. V., *O*-Acetyltransferases for chloramphenicol and other natural products. *Antimicrob. Agents Chemother.* 1997, *41*, 1 – 6.

[49] Duh, R. W., Singh, K. V., Malathum, K., Murray, B. E., *In vitro* activity of 19 antimicrobial agents against enterococci from healthy subjects and hospitalized patients and use of an *ace* gene probe from *Enterococcus faecalis* for species identification. *Microb. Drug Resist.* 2001, *7*, 39 – 46.

[50] Silverman, J., Thal, L. A., Perri, M. B., Bostic, G., Zervos, M. J., Epidemiologic evaluation of antimicrobial resistance in community-acquired enterococci. *J. Clin. Microbiol.* 1998, *36*, 830 – 832.

