Bundesinstitut für Risikobewertung

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Resistant bacteria: Wash uncooked vegetables and lettuce thoroughly and prepare them fresh by yourself

BfR Opinion No 013/2019 of 12 April 2019

Salads, fresh herbs and bean sprouts pre-cut for consumption and packed in plastic foil can contain bacteria that endanger your health. Despite preventive measures from cultivation all the way through to the point of sale, pathogens or antimicrobial resistant bacteria are still being detected in small numbers on fresh foods. Although this occurs less often than with meat, for example, any bacteria present in lettuce and fresh herbs are not inactivated by frying or cooking if these products are eaten raw.

The German Federal Institute for Risk Assessment (BfR) has assessed the latest results of research and food monitoring on the transfer of resistance to antibiotics through the consumption of fresh plant-based products. The assessment relates to a study conducted by the Julius Kühn Institute (JKI), which has already been published in a joint press release from the JKI and BfR (link, see Page 11). The focus was on *Escherichia coli* bacteria (*E. coli*), mostly harmless intestinal bacteria with a wide distribution which have also been detected in several raw salads, rocket and fresh coriander from the retail sector.

The tests showed that some *E. coli* bacteria are insensitive to several antibiotics, such as tetracycline, penicillins and cephalosporins. Once ingested with uncooked salad, basically harmless bacteria of this kind can pass on their resistance properties in the human gut to other pathogenic bacteria which may also exist there. As the resistance characteristics often lie on mobile genetic elements, they can be spread between *E. coli* as well as to other bacterial species. The extent to which a person is colonized permanently by resistant *E. coli* after eating raw vegetables cannot be estimated. It has to be assumed, however, that treatment with antibiotics at the time the bacteria are ingested with the salad could facilitate this process. Ingested resistant bacteria could also be the reason why treatment with antibiotics has no effect on a subsequent infection.

The BfR advises consumers to thoroughly wash raw vegetables, such as leaf lettuces and fresh herbs, with drinking water prior to consumption in order to minimise the risk of ingesting pathogens or antimicrobial resistant bacteria. Despite refrigeration, some bacteria can continue to propagate in the moist milieu of ready-to-eat mixed salad packagings, which are rich in nutrients, with the result that a slightly increased but still low risk of disease arises for particularly sensitive population groups. Pregnant women and people whose immune system has been weakened by age, previous illness or drug intake should therefore avoid eating pre-cut and packed salads as a precautionary measure to protect themselves from foodborne infections, and prepare salads made from fresh, thoroughly washed ingredients by themselves shortly before eating them.

Any pathogens or antimicrobial resistant bacteria which may exist on plant-based foods cannot be reliably removed through washing. It is therefore necessary in certain rare instances for particularly immunocompromised persons to heat vegetables and fresh herbs sufficiently (to a core temperature of 70° C for at least two minutes) prior to consumption in accordance with their doctor's instructions.

BfR Risk Profile: Illness through resistant bacteria in fresh, uncooked vegetables (Opinion No. 013/2019)						
A	**Affected groups**	**General population**				
B	**Probability of a health impairment through resistant bacteria in fresh, uncooked vegetables**	Practically excluded	**Unlikely**	Possible	Probable	Certain
C	**Severity of the health impairment through resistant bacteria in fresh, uncooked vegetables [1]**	**No impairment**	**Slight impairment [reversible]**	**Moderate impairment [reversible]**	Severe impairment [reversible/ irreversible]	
D	**Reliability of available data**	High: The most important data are available and free of contradiction	**Moderate: Some important data are missing or contradictory**	Low: Numerous important data are missing or contradictory		
E	**Controllability by the consumer [2]**	Control not necessary	**Controllable through precautionary measures**	Controllable through the uptake quantity	Not controllable	

Squares highlighted in dark blue indicate the properties of the risk assessed in this opinion (more detailed information on this is contained in BfR Opinion No. 013/2019 of 12 April 2019).

Explanations

The risk profile is intended to visualise the risk outlined in the BfR Opinion. It is not intended for the purpose of comparing risks. The risk profile should only be read in conjunction with the corresponding opinion.

[1] Line C – Severity of the health impairment
The severity of the impairment can vary depending on the type and quantity of ingested bacteria, the resistance properties of the bacteria towards antibiotics and the sensitivity of each person to the bacteria. The group of persons with particular susceptibility to foodborne infections includes pregnant women and people whose immune system has been weakened through advanced aged, previous illness or intake of drugs. In addition to this, it is likely that the colonisation of the human gut with resistant bacteria is facilitated by treatment with antibiotics.

[2] Line E – Controllability by the consumer
The information contained in the line “Controllability through the uptake quantity” should not be seen as a recommendation from the BfR but rather as having a more descriptive character. Recommendations can be found in the grey box on Page 1.

BUNDESINSTITUT FÜR RISIKOBEWERTUNG (BfR)

1 Object of the assessment

The German Federal Institute for Risk Assessment (BfR) has assessed current research results from the Julius Kühn Institute (JKI) on the transferable resistome with fresh products. The research results recently appeared in a publication produced by the American Society for Microbiology (Blau et al., mBio, November/December 2018, Volume 9, Issue 6, DOI: 10.1128/mBio.01300-18) and originate from a work group headed by Prof. Dr. Kornelia Smalla, Institute for Epidemiology an Pathogen Diagnostics at the JKI.
The BfR assessed the following individual aspects:

a) The methodical approach in the JKI publication and conclusions derived from it and
b) whether the currently valid behavioural and consumption recommendations for consumers have to be adapted due to the findings made in the publication.

2 Result

The results of the tests conducted at the JKI show that *E. coli* bacteria with tetracycline-resistance plasmids are to be found on raw lettuce, rocket and fresh coriander. Among the isolated *E. coli*, the scientists also identified a large number of different plasmids some of whose variable genomes had other resistant determinants in addition to the tetracycline-resistant gene. It was also possible to prove that the plasmids can be transferred between the bacteria fairly efficiently. Using a comparative test approach, it was shown that detection

methods with pre-enrichment of cultures result in more reliable detection of resistant bacteria than purely molecular biological methods (Blau et al., 2018).

The results of the tests are valid and confirm that resistant bacteria can occur on raw salads and fresh herbs. This has been known in principle for years, but the tests show the extent to which the resistance genes detected along with the bacteria found in the test occur on mobile genetic elements, thus making them fundamentally transferable between different species of bacteria.

Plant-based foods can transfer a large number of bacteria to humans. They are a possible source of infection with zoonotic pathogens, such as *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC) and *Listeria monocytogenes* (Söderqvist et al., 2017; Herman et al., 2015).

Bacteria of the species *E. coli* can be detected in a variety of processed and non-processed foods. Due to their ubiquitous distribution and the detection of *E. coli* in ready-to-eat foods, it has to be assumed that it is fundamentally possible to ingest the bacteria orally with food (Söderqvist et al., 2017; Alegbeleye et al., 2018).

The probability of the colonisation of humans with *E. coli* with a transferable, plasmid-coded antimicrobial resistance after the consumption of raw, contaminated plant-based foods cannot be conclusively estimated on the basis of the available data. The number of bacteria required to do so is not known. It is probable, however, that selection pressure caused by treatment with antibiotics favours colonisation with resistant bacteria as opposed to a situation without any selection pressure. Colonisation with the bacteria in itself is regarded as a risk factor for subsequent infection, but the genetic background of the *E. coli* along with host factors and other noxae play an additional, decisive role in the probability of an infection. A transfer of resistance plasmids to other bacteria (species), including pathogenic bacteria, is possible on the basis of the available research findings (Zeng & Lin, 2017).

There is no necessity, in the view of the BfR, to adapt the behavioural and consumption recommendations given to consumers on the basis of the new findings as these already take into consideration the possibility of the existence of resistant bacteria on plant-based foods if the bacteria have not been inactivated through heating. To protect against foodborne infections, the BfR advises people whose immune system has been weakened through pregnancy, advanced age, previous illness or drug intake to prepare salads consisting of fresh, thoroughly washed ingredients by themselves shortly before consumption. It would be better for these population groups to avoid eating pre-cut and packaged salads altogether. In general, consumers should wash raw vegetables, leaf lettuces and fresh herbs thoroughly in drinking water prior to consumption in order to minimise the risk of ingesting pathogens or antimicrobial resistant bacteria.

3 Justification

3.1 Assessment of the study

In the study by the work group headed by Prof. Dr. Smalla, a total of 24 samples of mixed salads, rocket and the spice plant coriander were purchased in German supermarkets and subsequently examined in a laboratory with cultivation-dependent and independent DNA-based methods. Using the cultivation-dependent approach, it was shown that *E. coli* which had transferable antimicrobial resistance plasmids with and without culture pre-enrichment could be detected on these foods (Blau et al., 2018). No quantitative information on the bacterial contamination of the examined samples is available, however.

The cultivation-independent examinations only produced partial evidence of resistance plasmids, even when highly specific and sensitive molecular biological detection techniques were used. Reliable detection was only possible after the enrichment of the bacteria.

The majority of the transferable plasmids have resistance factors for insensitivity towards tetracycline, ampicillin and amoxicillin. Additional genetic determinants were also identified which are known to trigger resistance to heavy metal compounds, sulphonamides, aminoglycosides and fluoroquinolones. Beta-lactam resistance genes of the type $bla_{CTX\text{-}M\text{-}1}$ which lead to the resistance of the bacteria to penicillins and cephalosporins of the 3rd generation were detected on three plasmids (Blau et al., 2018).

On the basis of the results presented, the JKI is recommending that persons who have to undergo treatment with antibiotics should be advised not to eat raw salads.

3.2 Risk assessment

3.2.1 Possible hazard source

Escherichia coli is a gram-negative rod ubiquitously distributed in the environment as a so-called faecal indicator, which means that it occurs in virtually all biological habitats. The majority of the bacteria of this species occur as harmless, nonpathogenic, commensal microorganisms, especially in the intestines of livestock, wild animals and humans. Several of the representatives of this species can show virulence factors which can cause mild to sometimes severe infections, which in turn transforms them into significant pathogens (NRC for nosocomial infections 2016). These include so-called Shiga toxin-producing *E. coli* (STEC), which can lead to gastroenteritic diseases as severe as haemolytic-uremic syndrome in humans in the form of enterohaemorrhagic *E. coli* (EHEC). A precise differentiation between apathogenic and pathogenic isolates is therefore essential for a valid assessment of the danger in addition to knowledge of the host factors.

Fundamentally, *E. coli* bacteria were detected in the gut of animals and humans in quantities of 10^8 to 10^9 colony-forming units (CFU)/g. Even though not all aspects of the significance of the bacterium in the gut of humans and animals are currently known, *E. coli* is fundamentally an important component of the natural microbiota of the intestinal tract which is involved in many different metabolic processes in the digestion of food (Muniesa et al., 2012).

Plasmids are mobile genetic elements which occur outside the bacterial host chromosome. They can be ingested and discharged via various mechanisms of horizontal gene transfer, but can also remain within the bacterium. Plasmids can contain a large variety of genetic information which can give their carrier additional properties (including antimicrobial resistance). Plasmids are very variably structured in their genetic composition and can be subject to strong evolutionary changes. The period of time or number of bacterial generations over which a plasmid of this kind can establish itself in a bacterium depends to a great extent on the actual plasmid type, additional plasmid-coded stabilisation systems (e.g. partitioning/addiction systems) and numerous other factors (Yang & Walsh, 2017). Moreover, the selection pressure can also be decisive for the colonisation of an ecosystem with resistant bacteria and/or the spread of resistance plasmids. Transferable plasmids play a special role here as they can either be self-transferable or mobilisable. Self-transferable (conjugative) plasmids possess all of the necessary factors to transfer their genome to other susceptible, usually closely related, bacteria. Contrary to this, mobilisable plasmids have to rely on a conjugative helper plasmid in order to be transferred. The plasmid transfer is often limited only to

closely related bacteria, whereby the efficiency of the transfer very much depends on the plasmid type (incompatibility group), the transfer system of the plasmid and the receiver bacterium (Dolejska & Papagiannitsis, 2018; Gama et al., 2018).

The plasmids from this study belong to different groups (incompatibility groups). Plasmids of these groups are described among other things with the occurrence of various resistance and transfer factors (Villa et al., 2010; Koraimann, 2018). It is not possible to make an estimation of the efficient transferability of the described plasmids on the basis of the available information. It also remains unclear to what extent the plasmids are transferable to other species of *Enterobacteriaceae* or other pathogens. To do so would require experimental tests in individual instances to detect this property of the plasmids beyond doubt.

Like many other representatives of *Enterobacteriaceae, E. coli* is also involved in the exchange of genetic information via mobile genetic elements (horizontal gene transfer). Transferable plasmids play a vital role here. In this way, the bacterium can acquire and pass on resistance genes which convey insensitivity to many representatives of the antimicrobial active substance classes relevant to human medicine, such as aminoglycosides and beta-lactam antibiotics, including the cephalosporins and carbapenems (e.g. meropenem). Experimental studies prove that some *E. coli* plasmids can be transferred very efficiently to other species within the *Enterobacteriaceae* family (Villa et al., 2010; Koraimann, 2018; Wang et al., 2013).

Resistance examinations of commensal *E. coli* isolates acquired from plant-based foods within the scope of national zoonosis monitoring showed that most of the isolates were fully sensitive to all test substances (Tab. 1). A few isolates showed resistance to several active substances. No *E. coli* were detected on the examined strawberries (485 samples, 2013), tomatoes (2016) and raspberries (2017) (BVL 2015, 2017, 2018).

Table 1: Microbial resistance to 14 antibiotics of *E. coli* obtained from plant-based foods using non-selective detection methods in the context of the zoonoses monitoring programme (BVL 2016a, 2016b, 2017).

Year of analysis	2012		2014		2015		2016	
Food	Leaf and cabbage lettuce		Fresh herbs		Pre-cut leaf lettuce		Sprouts	
No. of samples	756		381		360		357	
No. of received and analysed isolates[1]	14	% resistant	15	% resistant	9	% resistant	5	% resistant
Gentamicin	0	0	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0
Ceftazidime	0	0	0	0	0	0	0	0
Nalidixic acid	0	0	0	0	0	0	0	0
Ciprofloxacin	0	0	1	6.7	0	0	0	0
Ampicillin	0	0	3	20	2	22.2	0	0
Colistin	0	0	0	0	0	0	0	0
Sulfamethoxazole	0	0	2	13.3	1	11.1	0	0
Trimethoprim	0	0	1	6.7	1	11.1	0	0
Tetracycline	2	14.3	1	6.7	0	0	0	0
Azithromycin	n.a.[2]		0	0	0	0	0	0
Meropenem	n.a.		0	0	0	0	0	0
Tigecycline	n.a.		0	0	0	0	0	0
Sensitive	12	85.7	11	73.3	7	77.8	5	100
1x resistant	2	14.3	3	20	1	11.1	0	0
2x resistant	0	0	0	0	1	11.1	0	0
3x resistant	0	0	0	0	0	0	0	0
4x resistant	0	0	1	6.7	0	0	0	0
> 4x resistant	0	0	0	0	0	0	0	0

[1] Isolates were not received for every positive sample
[2] n.a.: not analysed

Selective tests for ESBL/AmpC-producing *E. coli* resulted in a detection rate of 2.2% in sprouts (2016) and fresh herbs (2014) and 2.3% in pre-cut leaf lettuce (2015), whereas the detection rate was zero in strawberries (2013), tomatoes (2016) and raspberries (2017) (BVL 2015, 2016a,b, 2017). And it was not only to 3rd generation cephalosporins that these isolates exhibited higher resistance rates than the non-selectively obtained isolates. Only a very small number of isolates were available, however, and quantification of the contamination of the foods was not possible due to the selected detection method (Table 2). The results of quantitative analyses of commensal *E. coli* are outlined in 3.2.3.

Table 2: Microbial resistance to 14 antibiotics of ESBL/AmpC-producing *E. coli* obtained from plant-based foods and identified using selective detection methods in the context of the zoonoses monitoring programme (BVL 2016a, b, 2017, BfR, unpublished data)

Year of analysis	2014		2015		2016	
Food	Fresh herbs		Pre-cut leaf lettuce		Sprouts	
No. of samples	405		381		361	
No. of received and analysed isolates[1]	5	% resistant	3	% resistant	5	% resistant
Gentamicin	1	20.0	0	0.0	2	40.0
Chloramphenicol	0	0.0	0	0.0	3	60.0
Cefotaxime	5	100.0	3	100.0	5	100.0
Ceftazidime	4	80.0	3	100.0	5	100.0
Nalidixic acid	1	20.0	1	33.3	3	60.0
Ciprofloxacin	1	20.0	2	66.7	5	100.0
Ampicillin	5	100.0	3	100.0	5	100.0
Colistin	0	0.0	0	0.0	0	0.0
Sulfamethoxazole	2	40.0	2	66.7	4	80.0
Trimethoprim	1	20.0	2	66.7	4	80.0
Tetracycline	2	40.0	2	66.7	4	80.0
Azithromycin	1	20.0	0	0.0	2	40.0
Meropenem	0	0.0	0	0.0	0	0.0
Tigecycline	0	0.0	0	0.0	0	0.0
Sensitive	0	0.0	0	0.0	0	0.0
1x resistant	0	0.0	0	0.0	0	0.0
2x resistant	2	40.0	0	0.0	0	0.0
3x resistant	0	0.0	1	33.3	1	20.0
4x resistant	2	40.0	1	33.3	0	0.0
5x resistant	1	20.0	1	33.3	0	0.0
6x resistant	0	0.0	0	0.0	2	40.0
7x resistant	0	0.0	0	0.0	1	20.0
8x resistant	0	0.0	0	0.0	1	20.0

[1] Isolates were not received for every positive sample

3.2.2 Hazard potential/Characterisation of hazard

Transmission of *E. coli* is mainly via the faecal-oral route, chiefly as a result of the consumption of contaminated foods (including water), direct contact with colonised animals or transmission between humans. It is above all the pathogenic representatives of the species *E. coli* (e.g. STEC/EHEC) that are of clinical significance, as they can sometimes cause severe clinical infections (Muniesa et al., 2012). Moreover, *E. coli* is of importance as a pathogen of nosocomial infections in health care establishments (National Reference Centre (NRZ) for Nosocomial Infections 2016).

When consumers ingest resistant bacteria with their food, the associated risks largely depend on the bacterium in question. Classic zoonotic pathogens (e.g. *Salmonella* spp., *Campylobacter* spp.) can directly result in illness, for example. If there is then a need for antibiotic therapy, antimicrobial resistance limits the therapeutic options. In the case of a food-associated infection caused by EHEC, the resistance properties of the pathogen play only a secondary role, as the therapeutic indication should be made with caution and as treatment does not as a rule take the form of antibiosis (Muniesa et al., 2012; Hagel et al., 2015).

In the case of facultative pathogenic bacteria, it is necessary to distinguish between colonisation with these bacteria and infections. With regard to commensal bacteria with resistance properties, there is currently no data on the dose that is required to colonise the intestinal tract of humans.

With regard to salmonellosis in humans, it has been shown that the intake of antibiotics increased the risk of disease (Mughini-Gras et al., 2014). The extent to which this also applies to colonisation with other resistant bacteria is not known, however. Previous antibiosis has been proven to have a protective effect against infections with *Campylobacter* spp. (Mughini-Gras et al., 2012). It was shown under clinical conditions that antibiotic therapy increased the risk of colonisation with specific resistant bacteria (Tacconelli et al., 2009), but the study in question did not specifically focus on intake via food. It is also known that previous antibiotic therapies increase the risk of becoming a carrier of ESBL/AmpC-producing *E. coli* (Karanika et al., 2016). Here again, though, it is unclear whether this increased colonisation rate is associated with the consumption of food.

It is not known which additional factors might play a role in determining whether a colonisation becomes an infection or whether there is any horizontal gene transfer. The prevalence of ESBL/AmpC-producing *E. coli*, for example, and the observed heterogeneity in the resistance genes, plasmids and bacteria in animals, foods and humans make it difficult to identify and reliably rule out specific connections (Madec & Haenni, 2018).

The submitted publication does not provide sufficient information to permit diagnostic classification of the plasmid-carrying isolates and their further genetic background (i.e. virulence factors), which means it is not possible to quantify the direct risk of disease resulting from these bacteria.

The variety of detected plasmids and their transmissibility creates major potential for an exchange of these genetic elements with other closely related bacteria. At the same time, however, the presence of the corresponding bacteria and mobile genetic elements does not on its own permit any assessment of the potential hazard for consumers. No information on transfer efficiency in the natural ecosystem is currently available. Neither can this information be deduced from the submitted study, as the tests were performed under optimised laboratory conditions. It is basically possible that bacteria with corresponding resistance factors ac-

cumulate in people undergoing antibiotic therapy due to the existing selection pressure and that the transmission rate of the resistance plasmids is increased (Zeng & Lin, 2017). It is further possible that transmissible plasmids are passed on to other bacteria and, where applicable, also to other pathogens. In the event of infections with these pathogenic bacteria, this could result in the limitation of therapeutic options.

3.2.3 Exposure

Escherichia coli can be detected in a wide range of plant-based foods. However, most studies have not characterised the detected *E. coli* in any greater detail, as these bacteria were either isolated as an indication of faecal contamination or to determine their resistance to antimicrobial substances. Some of the isolated *E. coli* showed a broad range of resistance to various classes of antimicrobial substances (Muniesa et al., 2012). The proportion of resistant isolates from this source was, however, far lower than in the *E. coli* that can be detected in foods like poultry meat, pork or veal (BVL 2016a,b, 2017).

Plant-based foods, in particular salads and herbs, are of special significance as they are often consumed without being heated, which means that any bacteria that are present are not reliably inactivated prior to consumption. The germ count of fresh fruit and vegetables is reduced by washing the produce with fresh drinking water. Above and beyond the findings outlined above, it can be assumed that the bacterial germ count on a plant-based food might be reduced even further by additional washing prior to consumption, thereby further reducing consumer exposure.

Based on the available information, it is not possible to exactly estimate the extent of exposure of the general population to transmissible resistance plasmids of *E. coli* or other *Enterobacteriaceae* through the consumption of plant-based foods. In view of the ubiquity and detection rates of *E. coli* in plant-based foods, however, it is to be assumed that oral intake of the bacterium with food is a possibility.

The study by the team headed by Prof. Dr. Smalla also showed that, despite the high sensitivity of DNA-based methods, it was only possible to detect resistance plasmids using culture-independent methods in individual cases. Detection was in some cases only performed using a culture-based method or using pre-enrichment. Molecular techniques are generally viewed as promising alternative methods for pathogen detection and often exhibit greater sensitivity and specificity (Rohde et al., 2017). The findings of the team under Prof. Dr. Smalla suggest that the level of contamination of the analysed plant-based foods with *E. coli* with transmissible resistance plasmids was low. This is confirmed by the results recorded by the national zoonoses monitoring programme. It has already been shown in multiple cases that plant-based foods sold in the retail sector had only very low levels of *E. coli* contamination. Using quantitative testing techniques, germ counts of *E. coli* were found in 4.5% of the analysed samples of fresh herbs (median: 210 CFU/g), 3.9% of the samples of pre-cut leaf lettuce (only 2/360 samples >1,000 CFU/g) and 4.8% of the samples of fresh sprouts (only 3/357 samples > 1,000 CFU/g) (BVL 2016a, b, 2017). In contrast, it was not possible to quantitatively determine any commensal *E. coli* in strawberries and frozen raspberries. *Escherichia coli* with transmissible resistance plasmids (ESBL-producing *E. coli*) was detected in roughly 2% of the tested plant-based foods (fresh herbs, pre-cut leaf lettuce and sprouts). These bacteria were not found in frozen raspberries. *Escherichia coli* isolates from plant-based foods showed fewer resistances than isolates from pork, veal or poultry meat across the board (BVL 2016a,b, 2017).

The bacterial counts of resistant *E. coli* measured in leaf lettuce indicate that any contamination is on the low side, but it is conceivable that the bacteria could multiply in packaged, pre-cut salads during the period until they are actually consumed.

3.2.4 Risk characterisation

The results of a study based on a small random sample (n = 24) submitted by the JKI confirm the findings of previous and more comprehensive studies which show that, when consuming raw salad and other raw vegetables as well as fresh herbs, humans are exposed to *E. coli* which can carry transmissible resistance properties (BVL 2016,a,b, 2017; Veldman et al., 2014; van Hoek et al., 2015). Where quantitative analysis was performed, however, the concentrations of the bacteria on the foods was very low in almost all cases. In the case of the study under assessment, it was only possible to reliably detect transmissible resistance plasmids after increasing the original bacterial concentrations (enrichment) and then obtaining the entire genetic material, and this also suggests low bacterial contamination with the relevant plasmids in the tested samples.

The necessary dose for colonisation of the human intestine with these bacteria is not known, but in view of the low observed bacterial count, the colonisation risk is assessed as present yet low. The risk that bacteria of this kind transfer their plasmids to other bacteria in the human intestine can be described as similarly low.

If consumers are simultaneously exposed to antimicrobial substances – as part of therapy with antibiotics, for example – it is likely that this favours the colonisation of the human intestine with these bacteria (Taconelli et al., 2009; Mughini-Gras et al., 2014). However, no reliable data are available for *E. coli* that would make it possible to assess the extent of the colonisation risk. The transfer rate of mobile genetic elements also increases under the influence of antibiotics (Zeng and Lin, 2017), which means that the transmission of resistance gene-carrying plasmids to other bacteria in the intestine would increase as a result of antibiotic treatment. Due to the low overall contamination of plant-based foods, however, it is considered unlikely that the consumption of raw plant-based foods would have any relevant effect on the health of healthy adults even if they are undergoing antibiotic treatment.

But even independently of the presence of transmissible resistance plasmids, it is still the case that certain particularly sensitive groups of consumers face an increased risk of illness due to the consumption of raw vegetable salads and fresh herbs.

4 Framework for action / Measures and recommendations

In order to avoid foodborne infections and intoxications as well as the transmission of resistance following the consumption of fresh produce such as salad and herbs, it is necessary to prevent them from being contaminated with human pathogens and resistant bacteria all the way from planting through to marketing by complying with the rules of Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP). The Codex Alimentarius has defined standards to this end. Moreover, in 2008 the WHO (World Health Organization) and the FAO (Food and Agriculture Organization of the United Nations) recommended comprehensive measures to reduce the contamination of fresh leaf products (FAO/WHO, 2008).

The BfR believes that, based on the test results published by the JKI, there is no need to alter the existing consumption recommendations for consumers.

Consumers should always wash raw vegetables, leaf lettuce and fresh herbs thoroughly with drinking water before eating them in order to minimise the risk of ingestion of pathogens or antimicrobial-resistant bacteria.

As certain bacteria can continue to breed due to the moist and nutrient-rich environment in pre-cut and packaged mixed salads despite cooling, particularly sensitive groups of people are exposed to a slightly increased – albeit still low – risk of illness following the consumption of pre-cut, packaged mixed salads. Pregnant women and people with compromised immune systems as a result of advanced age, pre-existing conditions or drug intake should therefore additionally refrain from eating pre-cut and packaged salads as a precaution against food-borne infections and should instead prepare salad themselves using fresh and thoroughly washed ingredients shortly before consumption.

However, washing alone may not be sufficient to reliably remove the disease pathogens or antimicrobial-resistant bacteria that may be present on plant-based foods. Therefore, in rare individual cases it is necessary that especially immunocompromised persons heat vegetables and fresh herbs sufficiently (to a core temperature of 70° C for at least two minutes) prior to consumption in accordance with their doctor's instructions.

Further information on resistance in plant-based foods on the BfR website:

"Resistant bacteria: Can raw vegetables and salad pose a health risk?", Press Release No. 40/2018 dated 8 November 2018
https://www.bfr.bund.de/en/press_information/2018/40/resistant_bacteria__can_raw_vegetables_and_salad_pose_a_health_risk_-207788.html

"Food made from leaves and grasses may contain pathogens", Press Release No. 28/2017 dated 10 July 2017
https://www.bfr.bund.de/en/press_information/2017/28/food_made_from_leaves_and_grasses_may_contain_pathogens-201349.html

BfR "Opinion app"

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