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RESEARCH

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Horizontal transfer of antibiotic resistance genes into microflora and blood cells in rats fed on GM-diet

Hanaa Abdel-Sadek Oraby*, Nadia Aboul-Ftooh Aboul-Maaty, Hayam Ahmad Al-Sharawi and Noha Mohamed Osman

Abstract

Background: With regard to the extensive production of genetically modified organisms, scientists focused on the safety of antibiotic resistance marker (ARM) genes present in GM-crops produced for the consumption of human or animal. We investigated the possible transfer of these genes to the microflora present in the gastrointestinal tract and blood cells of two groups of Male Wistar Albino rats fed on a transgenic diet containing the ARM genes *nptII* and *aadA* for 90 days. The conventional PCR was employed to screen for the presence of *nptII* and *aadA* genes in the experimental diets, and animals' samples.

Results: The occurrence of DNA transfer of *nptII* and *aadA* genes from GM-diet to blood and bacterial cells has been unambiguously demonstrated. Results were further confirmed by sequencing and blast analysis, indicating that both transferred segments shared significant alignment with number of Binary and Cloning Vectors with variable similarities. The frequency of transfer of DNA segments from the GM-diet into blood DNA was unexpectedly higher than its transfer to bacterial DNA which may be attributed to a number of factors.

Conclusions: Our results unambiguously demonstrated the occurrence of DNA transfer of ARM genes (*nptII* and *aadA)* from GM plant diet to blood cells and enteric microflora in rats. Results may draw attentions to the importance of exploring the possible effects of transfer of ARM genes horizontally from GM products to consumers and to extend our attentions to the importance of a better understanding of the factors influencing HGT in the intestine of the GM-food consumers.

Keywords: GMO, Horizontal gene transfer, Antibiotic resistance marker gens, *nptII*, aadA gene, Enteric microflora, Bacteria, Blood cells, PCR, Internal nucleotide sequencing

Background

Genetically modifying food technologies, like all new technologies, may have some known and unknown risks. Globally, great concern regarding genetically modified organisms (GMOs) is focusing on the safety of antibiotic resistance marker (ARM) genes in transgenic crops intended for human and animal consumption. Antibiotic resistance genes are known to produce enzymes to degrade antibiotics. Transfer of these genes to human or animal pathogens could make them resistant to available antibiotics (Bakshi 2003), which may, consequently, cause deleterious effects to public health.

The majority of antibiotic resistance genes used in biotechnology were isolated originally from bacteria (Miki and McHugh 2004). The neomycin phosphotransferase gene (*nptII*) conferring resistance to kanamycin and ampicilin is the most common selectable marker gene used in the transformation of various organisms as bacteria, yeast, plants and animals (Conner 1997). The

*Correspondence: haoraby@gmail.com

Department of Cell Biology, Biotechnology Research Institute, National Research Centre, 33 El Buhouth St., El Dokki, Cairo 12622, Egypt

source of *nptII* gene is the transposon (Tn5) from *E. coli* K12. The antibiotic hygromycin B is also used for selection as kanamycin (Miki and McHugh 2004). Resistance to hygromycin is confirmed mainly by aminoglycoside phosphotransferase (*aphIV*) which is also derived from *E. coli* (Day 2003; Miki and McHugh 2004). As for plastid transformation, the chimeric bacterial-derived antibiotic resistance marker, aminoglycoside 3″ adenyltransferase (*aadA*) gene, is the most widely used selectable marker. This gene (*aadA*) confers resistance to the antibiotics spectinomycin and streptomycin (Svab and Maliga 1993).

One of the hazards associated with the production of genetic modification is the probable spread of a DNA fragment from a donor cell/organism into unrelated recipient cells of another species through a process called horizontal gene transfer (HGT) (Keese 2008; Nielsen and Daffonchio 2007).

Several reports showed that transgenic or endogenous plant DNA could become incorporated into various tissues of animals (Oraby et al. 2014; Rizzi et al. 2012), leucocytes of rainbow trout (Chainark et al. 2008), goats (Tudisco et al. 2010), pigs (Mazza et al. 2005; Sharma et al. 2006) and human blood stream (Spisak et al. 2013), Mohr and Tebbe (2007) reported the transfer of segments of herbicide tolerance gene (pat-1) to gut bacteria in bees that pollinated GM Brassica. Another study (Netherwood et al. 2004) revealed evidence of low frequency transfer of a small fragment (180 bp) of EPSPS introduced gene derived from GM soybean to microorganisms within the small intestine of human ileostomists.

HGT of recombinant DNA from GMOs to bacteria is one of the unsettled biosafety issues (Nielsen et al. 2005). Scientists are concerned about transfer of such recombinant DNA which may promote the spread of antibiotic resistance (Beever and Kemp 2000; Chassy 2008; Ramessar et al. 2007). A study conducted by Woegerbauer et al. (2015) discovered that the *nptII*-load of the studied soils was low, rendering *nptII* a typical candidate as environmental pollutant upon anthropogenic release into these ecosystems.

Other studies (Wilcks and Jacobsen 2010) have suspected that DNA of genetically modified (GM) plants can be taken up by microbiota in the gut of animals fed on GM-plant diet. Chassy (2010) have also demonstrated that antibiotic resistance genes present in some transgenic crops have not added to the spread of antibiotic resistance in the environment.

The present work was planned to investigate the possible incorporation of transgenic DNA into enteric micro-flora of a group of experimental rats fed on GM-diet containing the antibiotic resistance marker (ARM) genes nptII and aadA for 90 days, using the polymerase chain reaction (PCR) assay. Transfer of ARM genes has also been investigated in blood cells of another group of experimental rats fed on the same GM-diet for the same duration (90 days). Samples were collected at three intervals (30, 60, and 90 days). The prevalence of the transferred ARM genes in blood cells of each of the experimental rats has been pursued all through the feeding duration. Further to confirm the transfer of these genes to the enteric microflora or blood cells, products of the PCR amplification of these genes were sequenced and analyzed through alignment with the GenBank Data Base.

Methods

Screening experimental diet samples for the presence of genetic modification

Animal feed samples were obtained from different animal feed suppliers in Cairo. None of these animal diets were labeled as genetically modified. Homogenous samples from each experimental diet were prepared by milling approximately 100–250 g of starting material. DNA was extracted from all samples applying a modified CTAB–based method (Aboul-Maaty and Oraby 2019).The presence of genetically modified contents in the purchased animal feed samples were investigated applying the conventional PCR assay, using primers for the Cauliflower Mosaic Virus -35S promoter (CaMVP-35S) and antibiotic resistance marker genes *nptII* and *aadA* (Table 1).

The internal nucleotide sequencing of the PCR amplification products of primers for P-35S and *nptII* and *aadA* genes has been performed by MWG-Biotech AG, to confirm the presence of CaMV 35S promoter and ARM genes *(nptII* and *aadA*) in the chosen animal feed sample.

Blast analysis with the GenBank was further performed for sequencing data to test for alignment.

The animal diet, which was experimentally proven to contain the targeted GM ingredients (P35S, *nptII* and *aadA*), was chosen for feeding the experimental rats during this investigation.

The potential for horizontal gene flow of ARM genes from GM-diet into enteric microflora and blood cells of experimental rats.

Animals, housing, and feeding durations

For bacterial analysis Male Wistar Albino rats (30 rats) obtained from the animal house of the National Research Centre shortly before weaning, were divided into three groups ten animals each. Group 1 was immediately euthanized (shortly before weaning) and considered as a control group (G1). The other two groups of animals were housed in standard cages and under standard ambient conditions for 30 days (G2) or 90 days (G3).The two experimental animal groups (G2 and G3) were euthanized at the end of the two feeding

Table 1 List of primers used throughout the experimental duration, their sequences, amplicon lengths and annealing temperatures

Primers	Positions on the genomes‡	Sequences (5′–3′)	Amplicon length (bp)	Annealing Temp (°C)	References
P-35S	7190–7209 7364–7384	5′-GCTCCTACAAATGCCATCA-3′ 5′-GATAGTGGGATTGTGCGTCA-3′	195	57	Hemmer (1997)
Cf3-Cr4	7313–7333 7411–7435	5′-CCACGTCTTCAAAGCAAGTGG-3′ 5′-TCCTCTCCAAATGAAATGAACTTCC-3′	123	62	Lipp et al. (2001)
NPTII	2382–2397 2554–2539	5′-GGATCTCCTGTCATCT-3′ 5′-GATCATCCTGATCGAC-3′	173	50	Hemmer (1997)
NPTII	2145–2167 2225–2245	5′-CTATGACTGGGCACAACAGACA-3′ 5′-CGGACAGGTCGGTCTTGACA-3′	101	60	Cited by Li et al. (2015)
aadA	1188–1208 1471–1451	5′-CGC TAT GTT CTC TTG CTT TTG-3′ 5′-TGA TTT GCT GGT TAC GGT GAC-3′	284	63	Clark et al. (1999), Hollingshead and Vapnek (1985)

‡ Positions of the primers are relevant to: Cauliflower mosaic virus genome (accession no. emb|V00141.1|), *NPTII* gene (accession no. AF080390.1), and *aadA* gene (accession no.MH973510.1)

Table 2 Blood sampling codes matrix

Animals' codes	Sampling codes			
	0-Day samples	Successive sampling times		
	Control	30 Days samples	60 Days samples	90 Days samples
A	A0	A30	A60	A90
B	B0	B30	B60	B90
C	C0	C30	C60	C90
D	D0	D30	D60	D90
E	E0	E30	E60	E90
F	F0	F30	F60	F90
G	G0	G30	G60	G90
H	H0	H 30	H 60	H 90
K	K0	K30	K60	K90

0-day samples are collected shortly before weaning; they represent control samples for the three successive collected samples from the same corresponding rat after GM-feeding durations of 30, 60, and 90 days

durations (after 30 and 90 days, respectively). The protocol applied during this study fulfills all the requirements of the NRC Ethical committee's guidelines (reference 12,142) as well as the ARRIVE guidelines, where all animals received humane care.

In order to study the potential for horizontal gene flow of antibiotic resistance marker genes from GM-diet into blood cells of investigated rats, an additional group of nine Male Wistar Albino rats were also purchased and were coded by the letters A, B, C, D, E, F, G, H, and K. A special approach was adopted for this part of our investigation, during which each animal is used as its own self-control (Table 2). Blood samples (1 ml each) were collected on EDTA (0.5%) from the coded experimental rats at four intervals. The first sample was collected shortly before weaning (i.e. 0-day sample) and before feeding them with the chosen GM-diet. Each of these samples (0-day samples) is used as a self-control for the three successive collected samples (after 30, 60, and 90 days) from the same corresponding rat (Table 2) during the whole feeding duration (90 days).

Sampling processing

Micro-flora in the rectum and caecal cavity of all animals groups were inoculated onto the surface of nutrient agar plates. These inoculated plates of all groups were incubated for 24–48 h at 37 °C, after which they were examined for colony characters, cellular morphology and the purity of the culture. Bacterial DNA extraction was performed by using GF-1 Bacterial DNA Extraction Kit (Vivantis) according to the manufacturer's procedure.

DNA was also extracted from blood samples collected from coded rats (Table 2) at the four intervals using DNA Purification Kit (Promiga Wizard Genomic DNA Purification Kit) following the manufacturer's manual. Determination of the quality and concentration of all DNA samples was performed using the Nano Drop 1000/ Thermo Scientific spectrophotometer.

The transfer of transgenic DNA from the GM-diet into the extracted DNA samples was tested using the conventional polymerase chain reaction (PCR) assay.

At least duplicates of most of the Polymerase Chain Reactions (PCR) were conducted in TM Thermal cycler (MJ Research PTC-100 thermocycler). PCR conditions and profile were carried out as described by Oraby et al. (2014, 2021).

The presence of a fluorescent band of the expected level for the investigated segments (*nptII* 173 bp-target, and *aadA* 284 bp-target) in all PCR products was analyzed using SYNGENE Bio Imaging Gel Documentation System. Further to confirm the obtained results, total fragment DNA purification kit (MEGA quick-spin,

iNtRON Biotechnology, Inc) was used for purification of some of these PCR products for the subsequent internal nucleotides sequencing analysis which was performed by MWG-Biotech AG. Following sequencing, BLASTN analysis with the GenBank was performed to test for alignment.

Results

Screening animal feed samples for the presence of genetic modification

The CaMVP35S promoter investigated in the present work (P-35S) was identified in all collected animal feed samples (1, 2, and 3) screened for the presence of genetic modification (Fig. 1). The figure also includes PCR products of amplified primers for *nptII* and *aadA* in diet samples.

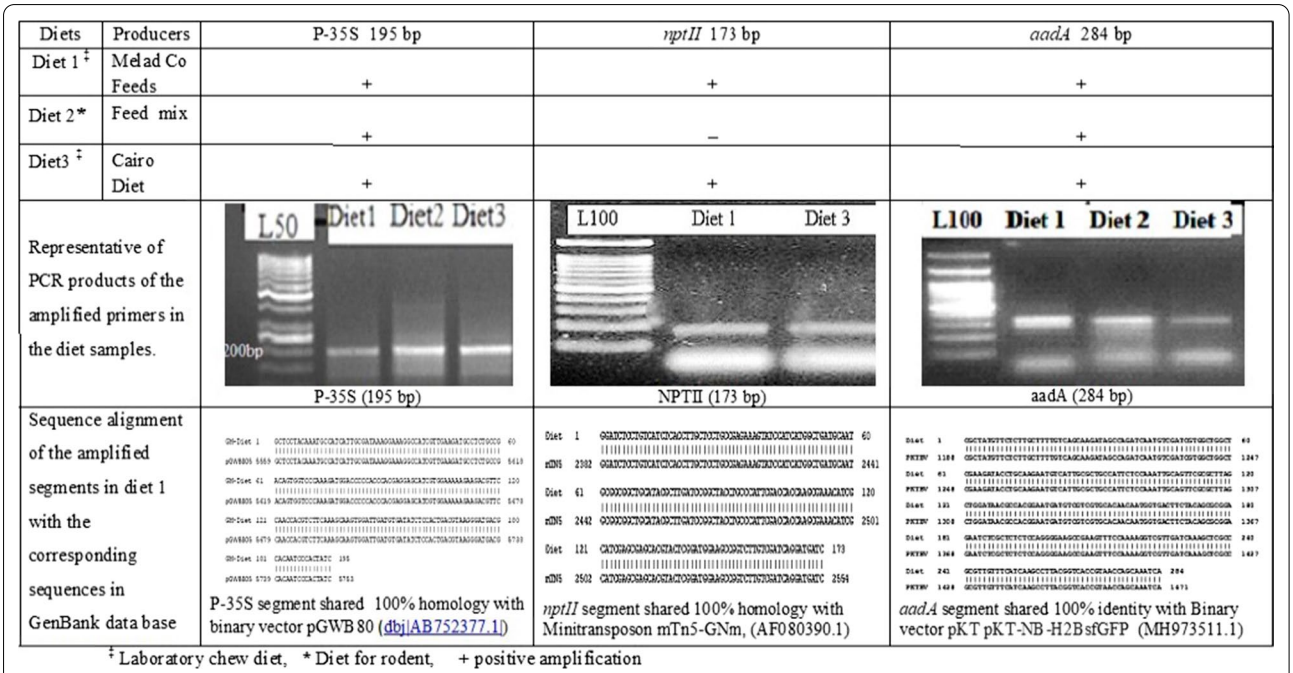
The presence of the amplified primers in DNA of the chosen animal feed (Animal diet 1) was further confirmed by DNA sequencing. The internal nucleotide sequencing analysis results of the PCR amplicon obtained from the amplification of primers for CaMV promoter (P-35S) and the antibiotic resistance marker genes *nptII* and *aadA* are included in the figure (Fig. 1), as well as results of Blast analysis with the GenBank. Alignment using NCBI-BLASTN program for the PCR amplicon of the investigated segments (P-35S, *nptII* and *aadA*) is also presented.

BLASTN analysis showed a 100% homology of the amplified segment with binary vector pGWB80 Acc. No. dbj|AB752377.1| at nucleotide (nt) coordinate 5559 to 5753. It also shared 100% sequence homology with CaMV-P35S promoter (emb|V00141.1|). The *nptII* retrieved segment (173 bp) shared 100% identity with Minitransposon mTn5-GNm, complete sequence (AF080390.1) at nt coordinate 2382–2554. The *nptII* retrieved segment also shared 100% identity with many other cloning, Binary and Gateway vectors. PCR amplification of the aadA primers gave a fragment of 284 bp in size. The *aadA* (284 bp) retrieved segment shared 100% identity with Binary vector pKT pKT-NB-H2Bsf-GFP, complete sequence (MH973511.1) at nt coordinate 1188–1471.

The potential for horizontal gene flow of ARM genes from GM-diet into enteric microflora and blood cells of experimental rats fed on GM-diet for three months

Bacterial DNA samples representing the three groups of animals were screened for the presence of CaMV 35S promoter, two segments of the antibiotic resistance marker gene *nptII* (101 bp-target and 173 bp-target) and a segment of the *aadA* gene(284 bp-target).

Results presented in Table 3 indicated that bacterial DNA extracted from GIT of the control group (G1) did not harbor any fragments from P-35S or from the antibiotic resistance marker genes (*nptII or aadA*). All tested primers showed no amplification in this group (G1). Table 3 also shows that tested primers for P-35S, *nptII*, and *aadA*, recorded variable amplification percentages



‡ Laboratory chew diet, * Diet for rodent, + positive amplification

Fig. 1 Detected genetic modifications in the collected diet samples and their nucleotide sequence alignment with GenBank

Table 3 Incidence of transfer of P35S, *nptII* and *aadA* into bacterial cells in GIT of rats fed on GM-diet

Bacterial DNA samples of control group of rats[1]					Bacterial DNA samples of the 30 days group					Bacterial DNA samples of the 90 days group				
Code	P35S 195 bp	*nptII* 101 bp	*nptII* 173 bp	*aad* 284 bp	Code	P35S 195 bp	*nptII* 101 bp	*nptII* 173 bp	*aad* 284 bp	Code	P35S 195 bp	*nptII* 101 bp	*nptII* 173 bp	*aad* 284 bp
C1	−	−	−	−	B1	+	−	+	+	B11	+	−	−	+
C2	−	−	−	−	B2	+	−	−	+	B12	+	+	+	+
C3	−	−	−	−	B3	−	+	−	+	B13	+	+	−	+
C4	−	−	−	−	B4	+	−	+	+	B14	+	−	+	+
C5	−	−	−	−	B5	+	+	+	+	B15	−	−	+	+
C6	−	−	−	−	B6	+	+	−	+	B16	−	+	+	+
C7	−	−	−	−	B7	+	+	+	+	B17	+	+	−	+
C8	−	−	−	−	B8	+	+	−	+	B18	+	+	−	+
C9	nt	−	−	−	B9	+	+	−	+	B19	+	+	−	+
C10	nt	−	−	−	B10	nt	−	−	−	B20	nt	−	−	+
%[P]					%[P]	88.8	60	40	90	%[P]	77.7	60	40	100

[1] Bacterial cells collected from control rats shortly before weaning

nt not tested, %[P]: percentage of transfer of GM sequences

in bacterial DNA extracted from GIT of the other two groups; G2 and G3 that were fed with GM-diet for 30 or 90 days, respectively. Primers for CaMV-35S promoter were amplified in about 89% and 78% of the bacterial samples in G2 and G3, respectively.

The primers pair for the smaller segment (101 bp) from *nptII* gene was amplified in 60% of each of the two investigated groups (G2 and G3), whereas primers pair for *nptII* gene (173 bp-target) was amplified in 40% of each of the two investigated groups (G2 and G3). Jointly, the two primers pairs for *nptII* gene (101 bp-target and 173 bp-target) recorded amplification percentage of 80% in each of the investigated groups G2 and G3.

Primers used for the amplification of segment from *aadA* gene (284 bp-target) were amplified in 90% and 100% of the bacterial cell in G2 and G3, respectively.

Sequencing and BLASTN analysis

It was not possible to obtain reliable sequencing results from the short segment (101 bp) of *nptII* gene. Therefore, in order to confirm the transfer of *nptII* fragment from the diet to microflora in GIT of rats fed on the GM diet for 90 days, the internal nucleotide sequencing of the amplified *nptII* longer segment (173 bp-target) was performed for two of these DNA samples (B14 and B15).

The retrieved sequences from both samples were 167 and 170 base pairs in size, respectively. Alignment of the two sequences (B14 and B15) revealed that similarity between the two sequences was only 96% (Fig. 2A).

Blast analysis for sequencing results (Fig. 2B) showed that the distribution of the top 101 Blast Hits on 100 subject sequences were the same for both samples B14 and B15, yet with variable similarities percentages (97% and 100%, respectively).

The internal nucleotide sequencing of the amplified *aadA* amplimers (284 bp-target) was performed for a number of samples from both groups, samples B3, B4, B7, B8 from G2 and samples B12, B13, B15, B16, B18, B20 from G3 group. The retrieved sequences from the amplified segments of the *aadA* gene were 284 base pairs in size. Clustal Omega multiple sequence alignment analysis for these sequences along with the corresponding segment from the reference sequence (MH973511.1), showed that they were not completely similar.

The internal nucleotide sequences similarities among the bacterial DNA samples ranged from 74.91 to 100.00% as presented in Fig. 2C, whereas similarity between them and the original reference sequence (MH973511.1) ranged from 82.27 to 99.29%.

Screening DNA of blood samples of experimental animals for the presence of ARM genes

The prevalence of DNA segment from GM-diet containing fragments from *nptII* (173 bp) and *aadA* genes in blood cells of investigated rats is presented in Table 4. Results revealed that no amplification occurred in DNA extracted from blood samples of rats collected shortly before weaning (0-day samples). Results also indicated that *nptII* was detected in all DNA of blood samples collected from rats fed on GM-diet after 30, 60 and 90 days except sample D90 in which *nptII* and *aadA* were not detected. PCR amplification of *nptII* (173 bp) fragment

A

```
B15     1    GGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT  60
             ||||||||||||||||| |||||||||| ||||||||||||||||||||||||||||||
B14     1    GGATCTCCTGTCATCTCACCTTGCTCCTGTCGAGAAAGTATCCATCATGGCTGATGCAAT  60

B15     61   GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG  120
             ||| |||  ||||| || ||||||||||||||||||||||||||||||||||||||||||
B14     61   GCG-CGG-GGCATA-GCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG  117

B15     121  CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATG  170
             |||||||||| ||||| ||||||||||||||||||||||||||||||||
B14     118  CATCGAGCGAGTACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATG  167
```

B

S. No.	Representative sample of sequences producing significant alignment with *nptII* (173 bp target)	Similarity % B14	Similarity % B15	Accession NO
1	Binary vector pCNM3	97	100	MG733987.1
2	Binary vector pCNF3	97	100	MG733986.1
3	Binary vector pCNRNF3	97	100	MG733985.1
4	Cloning vector pPKm-227, complete sequence	97	100	MG520652.1
5	Binary vector pK7WG_ASX, complete sequence	97	100	MG561371.1
6	Cloning vector pLXIN2, complete sequence	97	100	MF346346.1
7	Minitransposon mTn5-GNm, complete sequence	97	100	AF080390.1

C

Bacterial DNA Samples

B13	B13										
B3	71.91	B3									
B8	84.23	78.65	B8								
B18	86.43	84.45	93.26	B18							
B15	85.66	80.43	94.31	93.64	B15						
B20	86.69	81.07	96.43	95.39	96.10	B20					
B4	87.81	82.92	96.44	96.47	97.17	98.23	B4				
B12	88.89	82.92	95.02	95.76	96.47	96.81	98.59	B12			
B7	87.81	82.92	96.44	96.47	97.17	98.23	100.00	99.29	B7		
B16	88.17	82.56	96.09	96.11	96.82	97.87	99.65	98.94	99.65	B16	
OR	87.14	82.27	96.81	96.48	97.17	98.94	99.29	97.88	99.29	98.94	OR

Fig. 2 The internal nucleotide sequencing (for *nptII* and *aadA* amplimers), alignment and Blast analysis with gene bank. **A** Alignment of the internal nucleotide sequences of the amplified *nptII* in DNA of two of the microflora samples (B14 and B15) from GIT of rats fed on GM-diet for 90 days. **B** Variable similarities percentages of number of sequences that produced significant alignment with *nptII* in the two bacterial samples B14 and B15. **C** Percent Identity Matrix created by Clustal 2.1 for the internal nucleotide sequences of the PCR amplicons obtained from the amplification of primers for *aadA* gene in number of bacterial cells. OR is the original reference sequence of the corresponding segment of *aadA* (MH973511.1)

Table 4 Prevalence of transfer of *nptII* and *aadA* genes into blood cells of rats fed on GM-diet

Rats	Samples Codes and results of PCR amplification of *nptII* and *aadA* genes											
	0-Day samples[1]			30 Days samples[2]			60 Days samples[3]			90 Days samples[4]		
	Code	*nptII*	*aadA*	Code	*nptII*	*aadA*	Code	*nptII*	*aadA*	Code	*nptII*	*aadA*
A	A0	–	–	A30	+	+	A60	+	–	A90	+	–
B	B0	–	–	B30	+	+	B60	+	–	B90	+	+
C	C0	–	–	C30	+	+	C60	+	–	C90	+	–
D	D0	–	–	D30	+	–	D60	+	+	D90	–	–
E	E0	–	–	E30	+	+	E60	+	+	E90	+	+
F	F0	–	–	F30	+	+	F60	+	+	F90	+	+
G	G0	–	–	G30	+	+	G60	+	–	G90	+	+
H	H 0	–	–	H 30	+	–	H 60	+	–	H 90	+	+
K	K0	–	–	K30	+	–	K60	+	–	K90	+	+
%[P]				%[P]	100	66.6	%[P]	100	33.3	%[P]	88.8	66.6

[1] Blood samples collected from control rats just before weaning. [2]Blood samples collected from rats after being fed on the GM-diet for 30 days. [3]Blood samples collected from rats after being fed on the GM-diet for 60 days. [4]Blood samples collected from rats after being fed on the GM-diet for 90 days. %[P]: Percentage of transfer of GM sequences

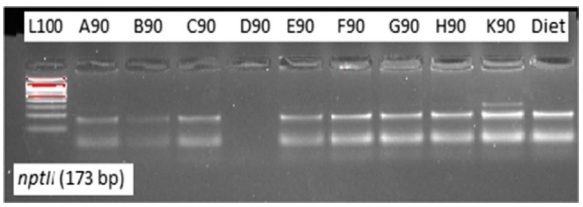


Fig. 3 PCR amplification products of *nptII* (173 bp) in blood samples of rats fed on GM-diet for 90 days

in blood samples collected from rats after 90 days is presented in Fig. 3.

The prevalence of DNA segments from GM-diet containing fragments from *aadA*(284 bp) gene in blood cells of investigated rats are also presented in Table 4. Results showed that *aadA* segment was not detected in DNA extracted from blood samples of rats collected shortly before weaning (0-day samples).

Results also showed that *aadA* investigated segment was eliminated from some of the blood DNA samples, after 90 days (A90, C90 and D90). These samples were harboring the *aadA* segment at earlier sampling times, (A30, C30, and D60, respectively).

Further to confirm the transfer of fragments from *nptII* and *aadA* genes from the GM-diet to the blood of rats, the internal nucleotide sequencing of the obtained *nptII* and *aadA* amplimers was performed. The internal nucleotide sequencing of the amplified *nptII* amplimers (173 bp-target) was performed for selected samples after 60 days (K60) and 90 days (C90, E90, F90 and G90). The retrieved sequences from these samples were 173 bp in size. Alignment of these five sequences using the Clustal Omega multiple sequence alignment program along with the corresponding segment from the reference sequence (AF080390.1) is presented in Fig. 4A.

CLUSTAL O (1.2.4) multiple sequence alignment (Fig. 4B) showed that samples C90, G90, F90 and K60 shared 100% identities with Minitransposon mTn5-GNm, complete sequence (AF080390.1). In case of E90, the identity percent was only 97.11%.

NCBI Blast analysis for NPTII (173 bp-target) sequencing results were carried out using BLASTN 2.9.0+ (Zhang et al. 2000). Blast analysis for C90, F90, K60, G90 internal nucleotide sequences gave 100% alignment with many cloning vectors (e.g. MK453498.1,MK448012.1, MK176935.1), Gateway binary vectors (e.g.AP018981.1, AP018954.1, AP018948.1) and other vectors (e.g. MH325111.1, MH325106.1, MK044344.1). Blast analysis for E90 internal nucleotide sequences gave 97.11% alignment with the same binary and cloning vectors.

Amplification of the *aadA* primers in DNA of blood samples of the experimental animals produced fragment of the size 284 bp. The internal nucleotide sequencing of the amplified *aadA* amplimers (284 bp-target) was performed for selected samples from the third sampling time group (E90, F90 and H90) fed on the GM-diet for 90 days.

Alignment of these sequences (E90, F90 and H90) using the Clustal Omega multiple sequence alignment program along with the corresponding segment from Binary vector pKT pKT-NB-H2BsfGFP and complete sequence (MH973511.1) are presented in Fig. 5A.

Results (Fig. 5B) showed that samples E90, F90 and H 90, respectively, shared 97.89%, 98.59 and 97.17% identities with the corresponding segment of *aadA* gene in the

A

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mTn5 2382   GGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT   2442
C90         GGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT   60
K60         GGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT   60
G90         GGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT   60
F90         GGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT   60
E90         GGATCTCCTGTCATCTCTCCTTGCTCCTGTCGAGAAAGTATCCATCATGGCTGATGCAAT   60
            ********************* *********** ******************************

mTn5 2443   GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG   2502
C90         GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG   120
K60         GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG   120
G90         GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG   120
F90         GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG   120
E90         GCGGCGGCTCCATACACTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCG   120
            ********* ***** ********************************************

mTn5 2503   CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATC   2555
C90         CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATC   173
K60         CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATC   173
G90         CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATC   173
F90         CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATC   173
E90         CATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTATCGATCAGGATGATC   173
            ************************************ ***************
```

B

	Tested samples					
mTn5	mTn5					
C90	100	C90				
F90	100	100	F90			
K60	100	100	100	K60		
G90	100	100	100	100	G90	
E90	97.11	97.11	97.11	97.11	97.11	E90

Fig. 4 Analysis of the results of amplification of *nptII* in blood cells of rats fed on GM-diet for 90 days. **A** Clustal Omega multiple sequence alignment of the internal nucleotide sequences of a number of blood samples along with the corresponding segment from the reference sequence mTn5 (AF080390.1). **B** Percent Identity Matrix—created by Clustal2.1—for the internal nucleotide sequences of the PCR amplicon (C90, F90, K60, G90 and E90) obtained from the amplification of primers for NPTII gene. mTn5 is the original sequence of the corresponding segment of mTn5 (AF080390.1)

A

```
OR/1188  cgctatgttctcttgcttttgtcagcaagatagccagatcaatgtcgatcgtggctggct  1248
F90      CGCTATGTTCTCTTGCTTTTGTCAGCAAGATAGCCAGATCAATGTCGATCGTGGCTGGCT  60
E90      CGCTATGTTCTCTTGCTTTTGTCAGCAAGATAGCCAGATCAATGTCGATCGTGGCTGGCT  60
H90      CGCTATGTTCTCTTGCTTTTGTCAGCAAGATAGCCAGATCAATGTCGATCGTGGCTGGCT  60
         ************************************************************

OR/1249  cgaagatacctgcaagaatgtcattgcgctgccattctccaaattgcagttcgcgcttag  1308
F90      CGAAGATACCTGCAAGAATGTCATTGCGCTGCCATTCTCCAAATTGCAGTTCGCGTTTAG  120
E90      CGAAGATACCTGCAAGAATGTCATTGCGCTGCCATTCTCCAAATTGCAGTTCGCGTTTAG  120
H90      CGAAGATACCTGCAAGAATGTCATTGCGCTGCCATTCTCCAAATTGCAGTTCGCGTTTAG  120
         ************************************************************ ****

OR/1309  ctggataacgccacggaatgatgtcgtcgtgcacaacaatggtgacttctacagcgcgga  1368
F90      CTGGATAACGCCACGGAATGATGTCGTCGTGCACAACAATGGTGACTTTTACAGCGCGGA  180
E90      CTGGATAACGCCACGGAATGATGTCGTCGTGCACAACAATGGTGATTTTTACAGCGTGGA  180
H90      CTGGATAACGCCACGGAATGATGTTGTGGTGCACAACAATGGTGACTTTTACAGCGTGGA  180
         ************************ ** ***************** ** ******* ***

OR/1369  gaatctcgctctctccaggggaagccgaagtttccaaaaggtcgttgatcaaagctcgcc  1428
F90      GAATCTCCCTCTATCCAGGGGAAGCCGAAGTTTCCAAAAGGTCGTTGATCAAAGCTCGCC  240
E90      GAATCTTCCTCTCTCCAGGGGAAGCCGAAGTTTCCAAAAGGTCGTTGATCAAAGCTCGCC  240
H90      GAATCTTCCTCTATCCAGGGGAAGCCGAAGTTTCCAAAAGGTCGTTGATCAAAGCTCGCC  240
         ******  **** ************************************************

OR/1429  gcgttgtttcatcaagccttacggtcaccgtaaccagcaaatca  1472
F90      GCGTTGTTTCATCAAGCCTTACGGTCACCGTAACCAGCAAATCA  284
E90      GCGTTGTTTCATCAAGCCTTACGGTCACCGTAACCAGCAAATCA  284
H90      GCGTTGTTTCATCAAGCCTTACGGTCACCGTAACCAGCAAATCA  284
         *********************************************
```

B

	Samples			
Original (MH973511.1)	Original			
F90	98.59	F90		
E90	97.89	98.59	E90	
H90	97.17	98.59	98.59	H90

C

BLASTN Alignment Results

Vectors	Acc.Number	Identity Percent for Samples		
		E 90	F 90	H 90
Binary vector pRE1, complete sequence	AY456904.1	97.89 %	98.59 %	97.54
Cloning vector pSKA417, complete sequence	MK775703.1	97.54 %	98.24%	97.18 %
Cloning vector AbVec6W-hIgG1, complete sequence	MK909077.1	97.54 %	98.24 %	97.18 %
Cloning vector AbVec6W-hIgLambda, complete sequence	MK909076.1	97.54 %	98.24 %	97.18 %
Cloning vector AbVec6W-hIgKappa, complete sequence	MK909075.1	97.54 %	98.18%	97.18 %
Cloning vector L1609, complete sequence	MH662439.1	97.54 %	98.18%	97.18 %

Fig. 5 Analysis of amplification results of *aadA* in blood cells of rats fed on GM-diet for 90 days. **A** Clustal Omega multiple sequence alignment of the internal nucleotide sequences of *aadA* (284 bp) in a number of blood samples along with the corresponding segment from the reference sequence (MH973511.1). **B** Percent Identity Matrix created by Clustal2.1 for the internal nucleotide sequences of the PCR amplicon (F90, E90, and H90) obtained from the amplification of primers for *aadA* gene. Original is the reference sequence of the corresponding segment of *aadA* (MH973511.1). **C** Variable similarities percentages of number of vectors that produced significant alignment with *aadA* amplemers

original Binary vector pKT pKT-NB-H2BsfGFP, complete sequence (MH9735111.1).

NCBI Blast analysis for *aadA* (284 bp-target) sequencing results was carried out using BLASTN 2.9.0+ (Zhang et al. 2000). Blast analysis for F90 and E90 and H90 internal nucleotide sequences resulted in alignment with number of Cloning Vectors, and Binary Vector with variable identity percentages as presented in Fig. 5C.

Discussion

Despite all of the benefits of GMOs, there is great concern among scientists about the possible occurrence of horizontal gene transfer of antibiotic resistance marker (ARM) genes, employed in the production of transgenic plants, into different tissues of animal or human consuming these GM products.

The present work explored the potential for horizontal gene flow of ARM genes from GM-diet into enteric microflora and blood cells of experimental rats fed on the laboratory chow diet for 90 days. As reported during the present study, the chosen laboratory diet sample used for this experiment gave positive results when screened for the presence of segments from CaMV-35S promoter, neomycin phosphotransferase II (*nptII*) gene and aminoglycoside 3″ adenyltransferase (*aadA*) gene. The presence of these segments was further confirmed by DNA sequencing and BLASTN analysis (Fig. 1). Antibiotic resistance marker genes are mostly introduced to GM plant as part of the bacterial vectors which are used for the initial gene constructions for transformation purposes. The *nptII* gene is usually used as a selectable marker in the initial laboratory stages to select plants that were genetically modified (Jelenic 2003; Turrini et al. 2015). The choice of the selective agent is important and based on the plant species to be transformed. However, *nptII* is probably the most widely used selectable marker for plant transformation, whereas *aadA* gene is usually used in the laboratory prior to the production of the genetically modified plants to select for bacteria containing the modified DNA.

In the present work, PCR results demonstrated the positive occurrence of DNA transfer of the antibiotic resistance marker genes *nptII* and *aadA* from GM diet to the enteric microflora (Table 3) and blood cells (Table 4) of experimental animals fed on the GM-diet for 90 days. These results were further confirmed by performing the internal nucleotide sequencing of the amplified *nptII* and *aadA* amplimers in number of DNA purified from the amplification products (amplicons). Sequencing was followed by alignment with the corresponding segments from the Gene Bank and further BLASTN analysis was performed to test for sequences similarities percentages (Figs. 2A–C). As presented in Fig. 2B, blast analysis with the gene bank for *nptII* sequences gave variable similarities percentages with many cloning and binary vectors. These results confirm the transfer of segments of *nptII* gene from the GM-diet into the bacterial cells of rats fed on this diet for 90 days.

Clustal Omega multiple sequence alignment among each of the incorporated genes (*nptII* or *aadA*) in bacterial cells samples showed that they were not completely similar. Alignment of *nptII* sequences showed that similarity was only 96% (Fig. 2A), where similarity among *aadA* sequences ranged from 74.91 to 100.00% (Fig. 2C). These similarity variations within bacterial DNA samples may point out to the possibility that the *aadA* chimeric gene is having high mutations rates, as in the case of bla recombinant gene which is characterized by having high mutations rates (Ho 2014).

The microbial system in the gastrointestinal tract (GIT) is very dynamic on the genetic level. It is capable of rapid response at the genetic level (Lerner et al. 2017). Its ecosystem is extremely enriched by mobile genetic elements (Feld et al. 2008; Bahl et al. 2004) that make it ideal for a potentially extensive gene exchange (Aminov 2011). Horizontal gene transfer (HGT) of antibiotic resistance genes is pervasive among prokaryotes, especially bacteria (Soucy et al. 2015).

The HGT of antibiotic resistance genes in the intestines of humans (Spisak et al. 2013) was reported. Its widespread in the human-associated microorganisms (Huddleston 2014; Liu et al. 2012; Smillie et al. 2011) was suggested to be due to the close physical proximity and increased cell-to-cell contact within the human body (e.g. gastrointestinal tract). Jeong et al. (2019) also suggested that 'phylogenetic effect' can significantly increase HGT activity among closely-related microorganisms. Transfer of these antibiotic resistance genes between these microorganisms, as suggested by Licht et al. (2003) may increase the possibility of acquisition of resistances by human pathogens through the use of antibiotics as additives in agricultural animal feed. Yau and Stewart (2013) revealed that the use of antibiotics may not only select for resistant populations but also may enhance the formation of new resistant strains by HGT.

On the contrary, transfer of transgenic DNA from the diet into the microflora found in GIT of rats fed on transgenic cucumber was not detected (Kosieradzka et al. 2001). However, as Nielsen and Townsend (2004) and Nielsen and BøhnT (2014) clarified that the different methodological approaches used have many limitations that may reduce the possibility to estimate the occurrence and impact of horizontal gene transfer in limited time study. Methodologically, they explained, the obtained bacteria from plate screening are only representing a

tiny proportion of the bacterial populations of the tested habitats.

The prevalence of the transferred ARM genes in blood cells of each of the investigated rats has also been pursued all through the feeding duration (90 days). As mentioned above, a special approach was adopted in this investigation, during which each animal, of the second group of rats, was used as its own self-control (Table 2). Therefore, each blood sample collected shortly before weaning was considered as control sample (0-day samples) for the other three samples collected from the same animal. Screening results showed that *nptII* and *aadA* segments were not detected in DNA extracted from blood samples collected from the animals shortly before weaning (0-day samples). These finding revealed that the control samples (0-day samples) were free from these segments (*nptII* and *aadA*) before the beginning of the feeding duration.

Results also showed that *nptII* was detected in all DNA of blood samples collected from rats fed on GM-diet for 30, 60 and 90 days except sample D90 in which *nptII* seemed to be eliminated (Fig. 3).

The *aadA* investigated segment was not detected in some of the blood DNA samples, after 90 days (A90, C90 and D90). In view of the fact that these *aadA* segments were detected in the same samples at earlier sampling times (A30, C30, and D60, respectively), suggests that these missing transferred segments seemed to be eliminated too from these samples. These findings imply that incorporation of both *nptII* and *aadA* segments were not within the nucleus of the blood cells at the earlier sampling times A30, C30 and D60 (Table 4), which is supported by the fact that a foreign genetic material usually must be incorporated in the nucleus of the recipient cell in order to be permanently added to eukaryotic cells (Moses 1987). Our findings also suggest the possible occurrence of a repair mechanism which may result in the elimination of the transferred segment at that latter stage of sampling time.

More than 80% of genetically modified plants (Cankar et al. 2008) contain the CaMVP-35S promoter, therefore, we investigated the transfer of a segment of P-35S (195 bp-target) into bacterial cells in rats fed for 30 and 90 days on the GM-diet. Table 3 demonstrates that the rate of transfer of P-35S (195 bp-target) was 88.8% and 77.7% in bacterial samples after 30 and 90 days, respectively. We also explored the transfer of another segment (Cf3Cr4, 123 bp-target) from CaMV-P35S promoter into blood cells of rats fed on the GM-diet for 90 days. Results (Fig. 6) indicated a 100% presence of this fragment in all DNA samples collected from rats fed on GM-diet for 90 days. In a previous study, Oraby et al. (2014) reported that ingested fragments from the CaMV-35S promoter (195 bp-target) were incorporated

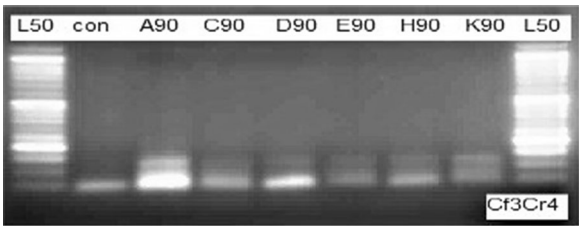

Fig. 6 PCR amplification of Cf3Cr4 in some blood samples of rats fed on GM-diet for 90 days. Cf3Cr4 (123 bp-target) is another segment from CaMV promoter, (con) is a negative control (0-day sample)

into blood, liver and brain tissues of experimental rats and the affinity of incorporation of different transgenic fragments from the ingested GM-diet into the different tissues of rats varied from one target sequence to the other. In some cases, only short DNA fragments derived from diet containing GM-maize were detected in blood lymphocytes of cows (Einspanier et al. 2001). Variation in rate of incorporation of the two investigated segments (101 bp-target and 173 bp-target) from *nptII* gene into bacterial cells were also reported in the present investigation.

Spisak et al. (2013) revealed that large meal-derived DNA fragments carrying a complete gene can avoid degradation and enter the human circulation system. Other studies reported the presence of DNA from M13 virus, GFP and even ribulose-1, 5- bisphosphate carboxylase (Rubisco) genes in the blood and tissue of experimental animals (Guertler et al. 2009; Brigulla and Wackernagel 2010).

Jelenic (2003) suggested that the presence of fragments from the diet-DNA harboring segments of CaMV promoter (P-35S, and Cf3Cr4) may cause a risk to the consumer. Studies conducted by Oraby et al. (2015a) suggested health hazards accompanying the ingestion of GM-diets containing CaMV promoter. They reported deleterious histopathological and histochemical impacts as well as biochemical alterations in different tissues of rats in association with the GM-diet consumption. Genotoxicity of the GM diet was previously reported in somatic and germ cells (Oraby et al. 2015b).

It has also been reported that CaMV-P35S promoter can function in a wide range of organisms (plant and animal). Expression of the bacterial *nptII* gene in Escherichia coli (Assaad and Signer 1999) can also be initiated by CaMV-P35S promoter. Despite the fact that, the bacterial promoter controlling the expression of *aadA* gene is not active in GM-plants (Moses 1987), yet CaMV-35S promoter sequence can alter the expression pattern not only in adjacent genes (Yoo et al. 2005), but also it can convert other specific gene promoter in

Table 5 Rates of transferred segments from the ingested GM-diet into blood or bacterial samples of rats after 90 days

Feeding period	Transferred segments	Blood samples				Bacterial samples			
		No. of samples	No. of samples with transferred segments	Rate of transfer (%)	Average of transfer (%)	No. of samples	No. of samples with transferred segments	Rate of transfer (%)	Average of transfer (%)
GM-diet for 90 days	NPTII (173 bp)	9	8	88.88	85,18	10	4	40	72.59
	aadA (284 bp)	9	6	66.66		10	10	100.00	
	CaMV-P*	9	9	100.00		9	7	77.77	

CaMV-P*: Two segments from the CaMV promoter were used; Cf3-Cr4 (123 bp) was amplified in blood and P-35S (195 bp) was amplified in Bacteria

the adjacent tissues and organs into a globally active promoter (Zheng et al. 2007).

It is well known that bacteria (Prokaryotes) lack an organized nucleus and usually accept new DNA more easily than other tissues (Moses 1987), in the present study, the collective rate of transfer (%) for both genes (*nptII* and *aadA*) were unexpectedly, higher in blood cells than that in the bacterial cells (Table 5). This may be explained knowing that horizontal gene transfer which occurs naturally in bacteria (OECD 2010), directs the bacteria to impose DNA repair mechanisms in order to adapt to changing environmental conditions and to generate genetic diversity without losing too much genomic stability (Fall et al. 2007). Transformation frequencies varied in association with the recipient strain and the position on the bacterial genome (Fall et al. 2007). Jelenic (2003) assumed the occurrence of a more or less significant transfer of foreign food DNA into some types of consumer's cells depending on the animal species and the type of food. He concluded that in both mammals and birds, transfer of food-derived DNA fragments into the resident microflora, in the GIT, has been indicated to occur in the presence of sites for homologous recombination, or when the exogenous fragments are part of replicating plasmids. This was the case in our results, where sequencing and BLAST analysis for the amplified *nptII* (173 bp-target) gene in DNA of blood or bacterial samples showed 97% alignment with some synthetic construct clone Sp. (e.g. MK371206.1 and MK371204.1). The other amplified *aadA* gene in DNA of bacterial samples showed alignment percents ranged from 88 to 96% with some plasmids from different bacterial strains complete sequences (e.g. CP031295.1, CP028419.1 and LN830952.1).

These results in its turn, confirm that these amplified segments were transferred into blood or bacterial cells horizontally from the GM-diet. Genetic engineering technology is known to depend on designing vectors for cloning and transferring genes involving artificially recombining and manipulating genes from unrelated species and their viral pathogens, which is known to improve and increase the probability for horizontal gene transfer and recombination (Ho et al. 1998).

Conclusions

The occurrence of DNA transfer of antibiotic resistance marker genes *nptII* and *aadA* from GM plant material to blood cells and enteric microflora has been unambiguously demonstrated in rats fed on GM-diet. These results may draw attentions to the importance of exploring the possible effects of transfer of ARM genes horizontally from GM products to consumers and to extend our attentions to the importance of a better understanding of the factors influencing HGT in the intestine of the GM-food consumers.

Abbreviations

GMO: Genetically modified organisms; GM-diet: Genetically modified diet; DNA: Deoxiribonucleic acid; *nptII*: Neomycin phosphotransferase gene; *aadA*: Aminoglycoside 3″ adenyltransferase gene; bp: Base pair; ARM: Antibiotic resistance marker gene; HGT: Horizontal gene transfer; GIT: Gastro-intestinal tract.

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Author contributions

HO made substantial contributions to conception, planning of the work, analysis, and interpretation of results and also involved in drafting the manuscript and revising it critically for important intellectual content, as well as gave the final approval of the version to be published. NA-M made substantial contributions to conception and design of the work, involved in conducting the practical section of the work, and also involved in drafting the manuscript. HEl-S involved in conducting the practical section for the assessment of HGT in blood cells of the experimental animals. NMO involved in performing culturing the bacterial cells from the GIT of experimental animals and extraction of DNA from Bacterial cells. Each author has participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final version of the manuscript.

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Availability of data and materials
Authors declare that all data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
The protocol applied during this study comply with the NRC Ethical committee's guidelines (reference 12142), and all animals received humane care.

Consent for publication
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References

Aboul-Maaty NAF, Oraby HAS (2019) Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Natl Res Cent 43:25. https://doi.org/10.1186/s42269-019-0066-1
Aminov RI (2011) Horizontal gene exchange in environmental microbiota. Front Microbiol 2:158. https://doi.org/10.3389/fmicb.2011.00158
Assaad FF, Singer ER (1999) Cauliflower mosaic virus P35S promoter activity in *Escherichia coli*. Mol Gen Genet (MGG) 223:517–520. https://doi.org/10.1007/BF00264462
Bahl MI, Sorensen SJ, Hansen LH, Licht TR (2004) Effect of tetracycline on transfer and establishment of the tetracycline-inducible conjugative transposon Tn916 in the guts of gnotobiotic rats. Appl Environ Microbiol 70:758–764. https://doi.org/10.1128/AEM.70.2.758-764.2004
Bakshi A (2003) Potential adverse health effects of genetically modified crops. J Toxicol Environ Health, Part B 6:211–225. https://doi.org/10.1080/15287390390155553
Beever DE, Kemp, (2000) Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. Nutrition abstracts and reviews. Ser B: Livest Feeds Feed 70:175–182
Brigulla M, Wackernagel W (2010) Molecular aspects of gene transfer and foreign DNA acquisition in prokaryotes with regard to safety issues. Appl Microbiol Biotechnol 86(1027–1):041. https://doi.org/10.1007/s00253-010-2489-3
Cankar K, Chauvensy-Ancel V, Fortabat MN, Gruden K, Kobilinsky A, Jana Z, Bertheau Y (2008) Detection of nonauthorized genetically modified organisms using differential quantitative polymerase chain reaction: application to 35S in maize. Anal Biochem 376:189–199. https://doi.org/10.1016/j.ab.2008.02.013
Chainark P, Satoh S, Hirono I, Aoki T, Endo M (2008) Availability of genetically modified feed ingredient: investigations of ingested foreign DNA in rainbow trout Oncorhynchus mykiss. Fish Sci 74:380–390. https://doi.org/10.1111/j.1444-2906.2008.01535
Chassy B (2008) Food safety of transgenic rice in rice improvement in the genomics era (Datta, S.K., ed.). Taylor and Francis, Abingdon
Chassy BM (2010) Food safety risks and consumer health. New Biotechnol 27:535–544. https://doi.org/10.1016/j.nbt.2010.05.018
Clark NC, Olsvik Ø, Swenson JM, Spiegel CA, Tenover FC (1999) Detection of a streptomycin/spectinomycinadenylyltransferase gene (aadA) in Enterococcus faecalis. Antimicrob Agents Chemother 43:157–160. https://doi.org/10.1128/AAC.43.1.157
Conner AJ (1997) Genetically engineered crops. Environmental and food safety issues. Miscellaneous series 39. The Royal Society of New Zealand, Wellington
Day A (2003) Antibiotic resistance gesen in transgenic plants: their origins, undesirability and technologies for their elimination from genetically modified crops. In: Stewart NC (ed) Transgenic plants: current innovations and future trends. Horizon Scientific Press, Wymondham, pp 111–156
Einspanier R, Klotz A, Kraft J, Aulrich K, Poser R, Schwagele F, Jahreis G, Flachowsky G (2001) The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. Eur Food Res Technol 212:129–134. https://doi.org/10.1007/s002170000248
Fall S, Mercier A, Bertolla F, Calteau A, Gueguen L, Perrière G et al (2007) Horizontal gene transfer regulation in bacteria as a 'spandrel' of DNA repair mechanisms. PLoS ONE 2:e1055. https://doi.org/10.1371/journal.pone.0001055
Feld L, Schjørring S, Hammer K, Licht TR, Danielsen M, Krogfelt K, Wilcks A (2008) Selective pressure affects transfer and establishment of a Lactobacillus plantarum resistance plasmid in the gastrointestinal environment. J Antimicrob Chemother 61:845–852. https://doi.org/10.1093/jac/dkn033
Guertler P, Paul V, Albrech C, Meyer HH (2009) Sensitive and highly specific quantitative real-time PCR and ELISA for recording a potential transfer of novel DNA and Cry1Ab protein from feed into bovine milk. Anal Bioanal Chem 393:1629–1638. https://doi.org/10.1007/s00216-009-2667-2
Hemmer W (1997) Foods derived from genetically modified organisms and detection methods. BATS-Report 2/97, Basel, Switzerland. www.bats.ch/abstr/297intro.htm
Ho MW (2014) Evolution by natural genetic engineering. Sci Soc 63:18–23
Ho MW, Traavik T, Olsvik O, Tappeser B, Howard V, von Weizsacker C et al (1998) Gene technology and gene ecology of infectious diseases. MicrobEcol Health Dis 10:33–39. https://doi.org/10.3402/MEHD.V10I1.7823
Hollingshead S, Vapnek D (1985) Nucleotide sequence analysis of a gene encoding a streptomycin/spectinomycin adenyltransferase. Plasmid 13:17–30. https://doi.org/10.1016/0147-619x(85)90052-6
Huddleston JR (2014) Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infect Drug Resist 7:167–176. https://doi.org/10.2147/IDR.S48820
Jelenic S (2003) Controversy associated with the common component of most transgenic plants kanamycin resistance marker gene. Food Technol Biotechnol 41:183–190
Jeong H, Arif B, Caetano-Anolles G, Kim KM, Nasir A (2019) Horizontal gene transfer in human-associated microorganisms inferred by phylogenetic reconstruction and reconciliation. Sci Rep 9:5953. https://doi.org/10.1038/s41598-019-42227-5
Keese P (2008) Risks from GMOs due to horizontal gene transfer. Environ Biosaf Res 7:123–149. https://doi.org/10.1051/ebr:2008014
Kosieradzka I, Sawosz E, Pastuszewska B, Szwacka M, Malepszy S, Bielecki W, Czumińska K (2001) The effect of feeding diets with genetically modified cucumbers on the growth and health status of rats. J Anim Feed Sci 10:7–12. https://doi.org/10.22358/jafs/70025/2001
Lerner A, Aminov R, Matthias T (2017) Intestinal dysbiotictransglutaminases are potential environmental drivers of systemic autoimmunogenesis. Front Microbiol 8:66. https://doi.org/10.3389/fmicb.2017.00066
Li X, Wu Y, Li J, Li Y, Long L, Li F, Wu G (2015) Development and validation of a 48-target analytical method for high-throughput monitoring of genetically modified organisms. Sci Rep 5:7616. https://doi.org/10.1038/srep07616
Licht TR, Struve C, Christensen BB, Poulsen RL, Molin S, Krogfelt KA (2003) Evidence of increased spread and establishment of plasmid RP4 in the intestine under sub-inhibitory tetracycline concentrations. FEMS Micrbiol Ecol 44:217–223. https://doi.org/10.1016/S0168-6496(03)00016-3
Lipp M, Bluth A, Eyquem F, Kruse L, Schimmel H, Van den Eede G, Anklam E (2001) Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs. Eur Food Res Technol 212:497–504. https://doi.org/10.1007/s002170000274
Liu J, Wu D, Ahmed A, Li X, Ma Y, Li T et al (2012) Comparison of the gut microbe profiles and numbers between patients with liver cirrhosis and healthy individuals. Curr Microbiol 65:7–13. https://doi.org/10.1007/s00284-012-0105-8
Mazza R, Soave M, Morlacchini M, Piva G, Marocco A (2005) Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Res 14:775–784. https://doi.org/10.1007/s11248-005-0009-5
Miki B, McHugh S (2004) Selectable marker genes in transgenic plants: applications, alternatives, and biosafety. J Biotechnol 107:193–232. https://doi.org/10.1016/j.jbiotec.2003.10.011
Mohr KI, Tebbe CC (2007) Field study results on the probability and risk of a horizontal gene transfer from transgenic herbicide-resistant oilseed rape

pollen to gut bacteria of bees. Appl Microbiol Biotechnol 75:573–582. https://doi.org/10.1007/s00253-007-0846-7
Moses B (1987) Gene transfer methods applicable to agricultural organisms. National Academies Press, Washington, DC.https://www.ncbi.nlm.nih.gov/books/NBK217998/
Netherwood T, Martin-Orue SM, O'Donnell AG, Gockling S, Graham J, Mathers JC, Gilbert HJ (2004) Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat Biotechnol 22:204–209. https://doi.org/10.1038/nbt934
Nielsen KM, Daffonchio D (2007) Unintended horizontal transfer of recombinant DNA. In: Traavik T, Ching LL (eds) Biosafety first: holistic approaches to risk and uncertainty in genetic engineering and genetically modified organisms. Tapir Academic Press, Trondheim
Nielsen KM, Townsend JP (2004) Monitoring and modeling horizontal gene transfer. Nat Biotechnol 22:1110–1114. https://doi.org/10.1038/nbt1006
Nielsen KM, Berdal KG, Kruse H, Sundsfjord A, Mikalsen A, Yazdankhah S, Nes I (2005) An assessment of potential long-term health effects caused by antibiotic resistance marker genes in genetically modified organisms based on antibiotic usage and resistance patterns in Norway. VKM-Report, Norwegian Scientific Committee for Food Safety, Oslo, Norway
Nielsen KM, Bøhn T, Townsend JP (2014) Detecting rare gene transfer events in bacterial populations. Front Microbiol. https://doi.org/10.3389/fmicb.2013.00415
OECD (2010) Guidance document on horizontal gene transfer between bacteria. Series on harmonisation of regulatory oversight in biotechnology no. 50, OECD Environment Directorate, Paris. http://www.oecd.org/biotrack/
Oraby HA, Kandil MH, Hassan AAM, Al-Sharawi HA (2014) Addressing the issue of horizontal gene transfer from a diet containing genetically modified components into rats tissues. Afr J Biotechnol 13:4410–4418
Oraby H, Kandil M, Shaffie N, Ghaly I (2015a) Biological impact of feeding rats with a genetically modified- based diet. Turk J Biol 39:265–275. https://doi.org/10.3906/biy-1406-61
Oraby H, Kandil MMH, Shaffie NM (2015b) Evaluating risks of genetically modified feed in Wistar albino rats: cytogenetic and histopathological studies. J Biol Nat 3:10–20
Oraby HA, Aboul-Maaty NAF, Al-Sharawi HA (2021) Exploratory and confirmatory molecular approaches to determine genetically modified status in different crops. Bull Natl Res Cent 45:196. https://doi.org/10.1186/s42269-021-00654-3
Ramessar K, Peremarti A, Gomez Galera S, Naqvi S, Moralejo M, Muñoz M, Capell T, Christou P (2007) Biosafety and risk assessment framework for selectable marker genes in transgenic crop plants. A case of the science not supporting the politics. Transgen Res 16:261–280. https://doi.org/10.1007/s11248-007-9083-1
Rizzi A, Raddadi N, Sorlini C, Nordgard L, Nielsen KM, Daffonchio D (2012) The stability and degradation of dietary DNA in the gastrointestinal tract of mammals: implications for horizontal gene transfer and the biosafety of GMOs. Crit Rev Food Sci Nutr 52:142–161. https://doi.org/10.1080/10408398.2010.499480
Sharma R, Damgaard D, Alexander TW, Dugan ME, Aalhus JL et al (2006) Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. J Agric Food Chem 54:1699–1709. https://doi.org/10.1021/jf0524590
Smillie CS, Smith MB, Friedman J, Cordero OX, David LA, Alm EJ (2011) Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480:241–244. https://doi.org/10.1038/nature10571
Soucy SM, Huang J, Gogarten JP (2015) Horizontal gene transfer: building the web of life. Nat Rev Genet 16:472–482. https://doi.org/10.1038/nrg3962
Spisak S, Solymosi N, Ittzes P, Bodor A, Kondor D, Vattay G, Bartak BK, Sipos F, Galamb O, Tulassay Z, Szallasi Z, Rasmussen S, Sicheritz-ponten T, Brunk S, Molnar B, Csabai I (2013) Complete Genes may pass from food to human blood. PLoS ONE 8(7):e69805. https://doi.org/10.1371/journal.Pone.0069805
Svab Z, Maliga P (1993) High-frequency plastid transformation in tobacco by selection for a chimeric aadA gene. Proc Natl Acad Sci USA 90:913–917. https://doi.org/10.1073/pnas.90.3.913
Tudisco R, Mastellone V, Cutrignelli MI, Lombardi P, Bovera F et al (2010) Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. Animal 4:1662–1671. https://doi.org/10.1017/S1751731116000409
Turrini A, Sbrana C, Giovannetti M (2015) Belowground environmental effects of transgenic crops: a soil microbial perspective. Res Microbiol 166:121–131. https://doi.org/10.1016/j.resmic.2015.02.006
Wilcks A, Jacobsen BB (2010) Lack of detectable DNA uptake by transformation of selected recipients in mono-associated rats. BMC Res Notes 3:49. https://doi.org/10.1186/1756-0500-3-49
Woegerbauer M, Zeinzinger J, Gottsberger RA, Pascher K, Hufnagl P, Indra A et al (2015) Antibiotic resistance marker genes as environmental pollutants in GMO-pristine agricultural soils in Austria. Environ Pollut (barking, Essex: 1987) 206:342–351. https://doi.org/10.1016/j.envpol.2015.07.028
Yau YY, Stewart CN (2013) Less is more: strategies to remove marker genes from transgenic plants. BMC Biotechnol 13:36. https://doi.org/10.1186/1472-6750-13-36
Yoo SY, Bomblies K, Yoo SK, Yang JW, Choi MS, Lee JS, Weigel D, Ahn JH (2005) The 35S promoter used in a selectable marker gene of a plant transformation vector affects the expression of the transgene. Planta 221:523–530. https://doi.org/10.1007/s00425-004-1466-4
Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203–214. https://doi.org/10.1089/10665270050081478
Zheng X, Deng W, Luo K, Duan H, Chen Y, McAvoy R, Song S, Pei Y, Li Y (2007) The cauliflower mosaic virus (CaMV) 35S promoter sequence alter the level and patterns of activity of adjacent tissue- and organ-specific gene promoters. Plant Cell Rep 26:1195–1203. https://doi.org/10.1007/s00299-007-0307-x

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