The Genotoxin Colibactin Shapes Gut Microbiota in Mice

CURRENT STATUS: POSTED

Sophie Tronnet
INSERM UMR1220 - IRSD

Pauline Floch
INSERM UMR1220 - IRSD

Laetitia Lucarelli
INSERM UMR1220 - IRSD

Deborah Gaillard
INSERM UMR1220 - IRSD

Patricia Martin
INSERM UMR1220 - IRSD

Matteo Serino
INSERM UMR1220 - IRSD

Corresponding Author
matteo.serino@inserm.fr
ORCID: https://orcid.org/0000-0003-4644-8532

Eric Oswald
INSERM UMR1220 - IRSD

DOI: 10.21203/rs.2.23021/v1

SUBJECT AREAS
General Microbiology

KEYWORDS
colibactin, gut microbiota dysbiosis, microbiome, enterobacteria
Abstract

Background: The genotoxin colibactin produced by resident bacteria of the gut microbiota may have tumorigenic effect by inducing DNA double strand breaks in host cells. Yet, the effect of colibactin on gut microbiota composition and functions remains unknown.

Results: To address this point, we designed an experiment in which pregnant mice were colonized with: i) a commensal E. coli strain, ii) a commensal E. coli strain plus a genotoxic E. coli strain, iii) a commensal E. coli strain plus a non-genotoxic E. coli mutant strain unable to produce mature colibactin. Then, we analysed the gut microbiota in pups at day 15 and day 35 after birth. At day 15, mice that were colonized at birth with the genotoxic strain showed lower levels of Proteobacteria and belonging taxa, a modest effect on overall microbial diversity and no effect on gut microbiome. At day 35, mice that received the genotoxic strain showed lower Firmicutes and belonging taxa, together with a strong effect on overall microbial diversity and higher microbial functions related to DNA repair. Moreover, the genotoxic strain strongly affected gut microbial diversity evolution of receiving pups between day 15 and day 35.

Conclusions: our data show that colibactin, beyond targeting the host, may also exercise its genotoxic effect on the gut microbiota.

Background

The quantitative and qualitative alterations, named dysbiosis, of the gut microbiota are now considered key traits of multiple pathologies such as metabolic, inflammatory and infectious diseases \[1\]. Dysbioses can originate from several factors related to multiple habits such as antibiotics abuse, among the strongest. However, an increased fat-to-fiber alimentary ratio, which is a common inducer of metabolic diseases, represents the strongest trigger of gut microbiota alterations. In this context, it was shown that a high-fat/high sucrose diet was able to increase the intestinal adherence of Adherent Invasive Escherichia coli (AIEC) in mice, thus promoting AIEC infection \[2\]. Both proinflammatory and genotoxic strains belong to Escherichia coli, which is a dominant member of phylum Proteobacteria and colonizes the gut of both humans and animals at birth \[3\]. Only 40% of the whole genome of E. coli is conserved, which confers to these bacteria a huge molecular plasticity. The latter is
characterized by the acquisition of mobile elements such as plasmids, transposons, phages and pathogenicity islands [4]. Genotoxic E. coli harbour in their genome a 52 kb polyketide synthase (pkS) pathogenicity island with genes encoding a complex enzymatic machinery synthetising the genotoxin colibactin. The B2 phylogenetic group of E. coli is the one harbouring the most (30%) of pkS+E. coli strains. The prevalence of the B2 group highly increased in the last decades and concomitantly with the progression of urbanization [5] and both autoimmune and allergic diseases [6]. This event induced a progressive passage from phylogenetic group A (with no pkS+E. coli strains) to phylogroup B2. Colibactin is capable of inducing DNA double strand breaks in eukaryotic cells [7] and of generating DNA interstrand cross-links [8] and was shown to induce multiple alterations in the host such as: cell senescence [9, 10], increased E. coli-induced lymphopenia [11], altered intestinal homeostasis [12], modified tumor microenvironment [13], colon tumor growth [9]. Yet, whether colibactin may exert its genotoxic effect even on members of the gut microbiota remains unknown. To address this point, we set-up a model of mother-to-pup vertical transmission of both genotoxic and non-genotoxic E. coli, since these strains are already present at birth[3]. We studied the effects on both gut microbiota and microbiome on pups at day 15 and 35 after birth. Beyond colibactin, pkS island genes are involved in the synthesis of multiple factors such as bacterial analgesic lipopeptide [14] and antibiotic molecules such as siderophore-microcins [15]. Given the strong importance of the mother as an E. coli transmitting factor [16], to get mice pups colonized by E. coli the most natural way, we decided to colonize the mother with the E. coli of interest. Therefore, to ascribe to the sole production of colibactin the putative effects on gut microbiota, we decided not to compare a pkS+ vs. a pkS- E. coli strain but, rather, to generate two isogenic bacteria mutants on the basis of the E. coli SP15 strain [11] and to colonize the mother with these mutants.

Results

**Colibactin targets the gut microbiota at the onset of intestinal colonization by genotoxic E. coli.**

To understand whether the genotoxin colibactin may also target host gut microbiota, beyond the effects observed on host cells[7, 8], we applied a protocol of vertical mother-to-pups transmission
(Supplementary Figure 1). Briefly, pregnant mothers were given by gavage either a non-genotoxic *E. coli* commensal strain (MG1655, phylogroup A, control group) or both MG1655 and the genotoxic *E. coli* SP15 strain (phylogroup B2, SP15clb+) or its non-genotoxic mutant (SP15clb-)). The molecular strategy applied to generate the two isogenic *E. coli* SP15 strains is reported in Supp.Fig.2. We analysed the overall putative changes in the gut microbiota of mice pups at 15 days after birth. The mother-to-pup transfer of the non-genotoxic *E. coli* SP15clb- strain was associated to a higher relative abundance of *Proteobacteria*. By contrast, the mother-to-pup transfer of the genotoxic *E. coli* SP15clb+ strain was associated to a higher relative abundance of family *Lachnospiraceae* (Fig.1A).

Both the non-genotoxic and the genotoxic *E. coli* SP15 strains significantly affected the overall gut microbiota profile (Fig.1B). However, the overall gut microbiota diversity was unaffected, despite the significant reduction in the Menhininck index, regardless of genotoxicity of the *E. coli* SP15 strain (Fig.1C). Then, considering the putative antibiotic activity of colibactin[17], we focused on microbial taxa whose abundance was lower following the colonization with the genotoxic *E. coli* SP15 strain. As reported in Fig.2A-F, the phylum- *Proteobacteria* and all the other related taxa displayed a significantly lower abundance in mice colonized with the genotoxic *E. coli* SP15clb+ strain compared to the non-genotoxic *E. coli* SP15clb- strain. Next, we analysed the gut microbiome by performing a PICRUSt[18]-based functional analysis. As reported in Fig.3A-B, we identified microbial pathways significantly enriched in the control and in the group of mice pups co-colonized with the non-genotoxic *E. coli* SP15clb- strain, but not with group co-colonized with the genotoxic *E. coli* SP15clb+ strain. Overall, these data show that 15 days after birth *E. coli* genotoxic activity exerts an intra-species taxonomical but not functional impact on the gut microbiota.

**Colibactin targets the gut microbiota and microbiome following intestinal colonization by genotoxic *E. coli***.

Then, to investigate whether the early impact of colibactin had long lasting consequences on gut microbiota composition, we analysed the overall putative changes in the gut microbiota of mice 35 days after birth. Differently from what observed above at day 15, the co-infection with the non-genotoxic *E. coli* SP15clb- strain was associated to a higher relative abundance of *Firmicutes*. By
contrast, the co-infection with the genotoxic SP15clb+ strain was associated to a higher relative abundance of genus *Alistipes* and family *Rikenellaceae* (**Fig.4A**). A PCA showed a complete separation between the gut microbiota profile of mice co-colonized with the genotoxic *E. coli* SP15clb+ strain compared to the the other groups of mice (**Fig.4B**). In addition, the calculation of several diversity indices showed a precise cluster separation among the three gut microbiota profiles, with a general significant higher diversity induced by the genotoxic strain compared to the non-genotoxic one, except for the Berger-Parker index (**Fig.4C**). As for the reduction in microbial taxa the phylum *Firmicutes* and all the other related taxa displayed a significantly lower abundance in mice colonized with the genotoxic *E. coli* SP15clb+ strain compared to the non-genotoxic *E. coli* SP15clb- strain (**Fig.5A-F**). Next, we analysed the gut microbiome by performing a PICRUST[18]-based functional analysis. As reported in **Fig.6A-B**, we identified microbial pathways significantly enriched in all the three groups. In detail, microbial pathways related to replication and repair, DNA repair and recombination proteins and DNA replication, among the totality identified, were found significantly enriched in the mice co-colonized with the genotoxic *E. coli* SP15clb+ strain (**Fig.6B**).

Overall, these data show that 35 days after birth, the *E. coli* genotoxic activity profoundly affect the gut microbiota at a both taxonomical and functional level and that it exerts an inter-species impact on the gut microbiota.

**Evolution of gut microbiota and microbiome following intestinal colonization by genotoxic or non-genotoxic *E. coli* SP15 strain in mice.**

Next, we evaluated the effects of the infection with the above reported *E. coli* strains on the evolution of gut microbiota and microbiome, by comparing the two time points at day 15 and day 35 after birth. On a taxonomical level, mice that received *E. coli* MG1655 strain displayed increased abundance of the family *Lachnospiraceae* at day 35 after birth (**Suppl.Fig.3A**); in terms of overall diversity, the infection with *E. coli* MG1655 did not induce a net separation between the two time points of 15 and 35 days (**Suppl.Fig.3B**), albeit some microbial functions were found significantly enriched (**Suppl.Fig.3C**). Mice that received the non-genotoxic *E. coli* SP15clb- strain displayed increased abundance of the phyla *Firmicutes* and *Deferribacteres* at day 35 after birth (**Suppl.Fig.4A**). The
overall microbial diversity between the two time points of day 15 and day 35 was not affected (Suppl.Fig.4B), albeit some microbial functions were found significantly enriched (Suppl.Fig.4C). By contrast, mice that received the genotoxic E. coli SP15clb+ strain displayed increased level of the phyla Deferrribacteres and Tenericutes at day 35 after birth (Suppl.Fig.5A) and a net microbial diversity separation between the two time points of dy 15 and day 35, with cellular processes related to signalling as the identified microbial function found significantly enriched (Suppl.Fig.5C). Overall, these data show that the genotoxic E. coli SP15clb+ strain affected the gut microbiota diversity to a greater extent compared to the other E. coli strains.

Discussion
In this study we report the effects of the genotoxin colibactin on both gut microbiota and microbiome in mice pups whose mothers have been colonized with either a genotoxic or a non-genotoxic E. coli strain. We adopted this protocol to follow the natural mother-to-pup vertical transmission, which is among the strongest factor for E. coli dissemination in further generations [16]. The genotoxin colibactin was demonstrated to act on eukaryotic cells by affecting DNA stability [7, 8] and to affect the host at multiple levels [9–13]. These effects are likely to be blunted by an efficient intestinal mucus barrier, since an adherent mucus layer on epithelial cells was shown to dampen colibactin-induced DNA double strands breaks in vitro [19].

It was recently reported that another genotoxin, the cytolethal distending toxin produced by the human clinical isolate C. jejuni81-176, may affect both the microbial composition and gene expression profile of the gut microbiota of 1% dextran sulfate sodium-fed germ-free (GF) ApcMin/+ mice colonized with C. jejuni81-176 [20]. Beyond these evidences, we investigated whether colibactin may also exert its action against other bacteria of the gut microbiota, which was not known. Our data show a time-dependent double targeting action on gut microbes: i) 15 days after birth, the maternally acquired E. coli genotoxic strain appears to exert an intra-species effect on gut microbes, by targeting bacteria from taxa belonging to the phylum Proteobacteria, to which E. coli belongs; ii) then, 35 days after birth, the action of colibactin appears directed against bacteria from Proteobacteria-unrelated taxa of the gut microbiota, such as those belonging to the phylum Firmicutes, showing an inter-
species effect. Therefore, our data showing that colibactin may also target the gut microbiota support the putative antibiotic activity of colibactin [17], which may first be devoted to help genotoxic bacteria creating their own niche. Then, once the niche created, the genotoxic activity of bacteria may help them expanding their niche by targeting gut microbes of unrelated taxa. This interpretation is also supported by the effects of colibactin we observed on the gut microbiome at day 35 after birth, where microbial pathways related to DNA repair were the most affected. This evidence is in line with the capacity of colibactin to affect DNA stability [7, 8] and thus induce the DNA repairing machinery. In terms of the evolution of the gut microbiota, the genotoxin colibactin strongly changed the overall microbial diversity from day 15 to day 35 in mice which received the genotoxic E. coli strain, compared to mice which received the non-genotoxic E. coli strain.

Conclusion
Our data suggest that, in mice, the bacterial genotoxin colibactin may exert its activity even against the gut microbiota, beyond its known effects on the host [7, 9]. The implication of this evidence may help understanding the benefits that the acquisition of genotoxicity may provide to pks + bacteria, such as the establishing and expanding of their own niche, within the complex microbial ecosystem which is the intestine.

Methods

Animal model and tissue collection
The animal model used in this study was already published[12]. Briefly, primiparous timed-pregnant Swiss CD1 mice were purchased from Janvier labs, housed separately under specific-pathogen-free conditions with access to food and water supplemented with streptomycin (5g/L) ad libitum. Pregnant females were inoculated once with $10^9$ bacteria by intragastric gavage the day before parturition. Mice were sacrificed in a fed state by cervical dislocation; then, caecal tissues were collected and snap-frozen in liquid nitrogen. All animal experimental procedures were approved by the local ethical committee (protocol n° CE2017031317082461V3) of Purpan University Hospital (Toulouse, France). All experiments were performed in accordance with relevant guidelines and regulations.

Strain construction
*E. coli* strain SP15 is an extraintestinal pathogenic *E. coli* (ExPEC) strain of serotype O18:K1 isolated from a patient with neonatal meningitis [21]. Strain SP15 harbors the pks island and was previously shown to produce colibactin [22]. Gene inactivation of clbP was engineered by using the lambda Red recombinase method [23] using primers clbP-P1 and clbP-P2 (Fig.S2) followed by excision of the kanamycin resistance cassette, as previously described [23], to produce strain SP15Δclb-. The functional wild-type clbP gene and the clbP gene that was site directed mutagenized to inactivate the catalytic site of the ClbP enzyme were PCR amplified from vectors pBRSK-clbP and pBRSK-clbP-S95A[24], respectively, using primers clbP-F-Bam and pBRSK-F-Bam (Fig.S2). The resulting PCR products were restricted by BamHI and cloned into vector pCM17 [25] digested by BamHI. The resulting plasmids pCM17-clbP and pCM17-clbP-S95A were transformed into strain SP15Δclb-. The resulting strains SP15clb+ and SP15clb- were demonstrated to produce and to not produce colibactin, respectively.

**Taxonomic analysis of the caecum microbiota by MiSeq**

Total DNA was extracted[26] from caeca at both day 15th and day 35th after birth. The 16S rRNA gene V3-V4 regions were targeted by the 357wf-785R primers and analyzed by MiSeq at RTLGenomics (http://rtlgenomics.com/, Texas, USA). An average of 19,825 sequences was generated per sample. A complete description of the applied bioinformatic filters is available at http://www.rtlgenomics.com/docs/Data_Analysis_Methodology.pdf. Cladograms were drawn by the Huttenhower Galaxy web application (http://huttenhower.sph.harvard.edu/galaxy/) via the LEfSe algorithm [27].

**Statistical analysis**

Results are presented as mean±SEM. Statistical analyses were performed by 2-ANOVA followed by a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli to correct for multiple comparisons by controlling the False Discovery Rate (<0.05) or Kruskal-Wallis test plus a two-stage step-up method of Benjamini, Krieger and Yekutieli correction for multiple comparisons by controlling the False Discovery Rate (<0.05) or Mann-Whitney test, as indicated in the figure legend, by using GraphPad Prism version 7.00 for Windows Vista (GraphPad Software, San Diego, CA). Significant
values considered when \(P<0.05\) or as reported after corrections. For cladograms, the alpha value for the factorial Kruskal-Wallis test among classes and the alpha value for the pairwise Wilcoxon test between subclasses have been changed to \(P<0.01\), as shown on figures. Principal component analyses were drawn and diversity indices calculated with the software PAST3, version 3.19 (https://folk.uio.no/ohammer/past/). Heat-maps based on a Pearson distance and a complete linkage were drawn with the PermutMatrix software[28].

Supplemental Information Note

**Figure S1.** Time-line of the experimental protocol employed in the co-infection model of vertical mother-to-pup transmission. **Figure S2.** Molecular strategy used to generate the non-genotoxic and genotoxic E. coli SP15 strains. **Figure S3.** 15-to-35 days after birth caecum microbiota evolution of mice pups which received MG1655 *E. coli*. **Figure S4.** 15-to-35 days after birth caecum microbiota evolution of mice which received MG1655 and *E. coli* SP15clb- strains. **Figure S5.** 15-to-35 days after birth caecum microbiota evolution of mice which received MG1655 and *E. coli* SP15clb+ strains.

Declarations

**Acknowledgements**

We thank the zootechnie-Purpan INSERM/UPS US006 CREFRE for excellent mouse managing. We thank Prof. Kaper JB and Ms. Jane Michalski Wilhelm for kindly providing plasmid pCM17.

**Authors’ contributions**

S.T., L.L., D.G. and P.M. performed mouse experiments; P.F. and M.S. extracted DNA from murine caeca; M.S. analysed gut microbiota and microbiome, prepared figures and wrote the manuscript; E.O. conceived and supervised the study. All authors gave final approval of the version to be published.

**Funding**

ANR-17-CE35-0010 to EO.

**Competing interests**

The authors declare that no conflict of interest exists.

**Author details**
Availability of data and materials. All data are available in the main text or the supplementary materials and via the following repositories: Sequence Read Archive (SRA) database https://submit.ncbi.nlm.nih.gov/subs/sra/ with the assigned identifier PRJNA593936.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Serino M: Molecular Paths Linking Metabolic Diseases, Gut Microbiota Dysbiosis and Enterobacteria Infections. J Mol Biol 2018, 430(5):581-590.

2. Agus A, Denizot J, Thevenot J, Martinez-Medina M, Massier S, Sauvanet P, Bernalier-Donadille A, Denis S, Hofman P, Bonnet R et al: Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation. Sci Rep 2016, 6:19032.

3. Secher T, Brehin C, Oswald E: Early settlers: which E. coli strains do you not want at birth? Am J Physiol Gastrointest Liver Physiol 2016, 311(1):G123-129.

4. Croxen MA, Finlay BB: Molecular mechanisms of Escherichia coli pathogenicity. Nat Rev Microbiol 2010, 8(1):26-38.

5. Tenaillon O, Skurnik D, Picard B, Denamur E: The population genetics of commensal Escherichia coli. Nat Rev Microbiol 2010, 8(3):207-217.

6. Bach JF: The effect of infections on susceptibility to autoimmune and allergic
diseases. *N Engl J Med* 2002, *347*(12):911-920.

7. Nougayrede JP, Homburg S, Taieb F, Boury M, Brzuszkiewicz E, Gottschalk G, Buchrieser C, Hacker J, Dobrindt U, Oswald E: **Escherichia coli induces DNA double-strand breaks in eukaryotic cells.** *Science* 2006, *313*(5788):848-851.

8. Bossuet-Greif N, Vignard J, Taieb F, Mirey G, Dubois D, Petit C, Oswald E, Nougayrede JP: **The Colibactin Genotoxin Generates DNA Interstrand Cross-Links in Infected Cells.** *MBio* 2018, *9*(2).

9. Cougnoux A, Dalmaso G, Martinez R, Buc E, Delmas J, Gibold L, Sauvanet P, Darcha C, Dechelotte P, Bonnet M et al: **Bacterial genotoxin colibactin promotes colon tumour growth by inducing a senescence-associated secretory phenotype.** *Gut* 2014, *63*(12):1932-1942.

10. Secher T, Samba-Louaka A, Oswald E, Nougayrede JP: **Escherichia coli producing colibactin triggers premature and transmissible senescence in mammalian cells.** *PLoS One* 2013, *8*(10):e77157.

11. Marcq I, Martin P, Payros D, Cuevas-Ramos G, Boury M, Watrin C, Nougayrede JP, Olier M, Oswald E: **The genotoxin colibactin exacerbates lymphopenia and decreases survival rate in mice infected with septicemic Escherichia coli.** *J Infect Dis* 2014, *210*(2):285-294.

12. Payros D, Secher T, Boury M, Brehin C, Menard S, Salvador-Cartier C, Cuevas-Ramos G, Watrin C, Marcq I, Nougayrede JP et al: **Maternally acquired genotoxic Escherichia coli alters offspring's intestinal homeostasis.** *Gut Microbes* 2014, *5*(3):313-325.

13. Dalmaso G, Cougnoux A, Delmas J, Darfeuille-Michaud A, Bonnet R: **The bacterial genotoxin colibactin promotes colon tumor growth by modifying the tumor microenvironment.** *Gut Microbes* 2014, *5*(5):675-680.
14. Perez-Berezo T, Pujo J, Martin P, Le Faouder P, Galano JM, Guy A, Knauf C, Tabet JC, Tronnet S, Barreau F et al: **Identification of an analgesic lipopeptide produced by the probiotic Escherichia coli strain Nissle 1917.** Nat Commun 2017, **8**(1):1314.

15. Massip C, Branchu P, Bossuet-Greif N, Chagneau CV, Gaillard D, Martin P, Boury M, Secher T, Dubois D, Nougayrede JP et al: **Deciphering the interplay between the genotoxic and probiotic activities of Escherichia coli Nissle 1917.** PLoS Pathog 2019, **15**(9):e1008029.

16. Mackie RI, Sghir A, Gaskins HR: **Developmental microbial ecology of the neonatal gastrointestinal tract.** Am J Clin Nutr 1999, **69**(5):1035S-1045S.

17. Fais T, Cougnoux A, Dalmasso G, Laurent F, Delmas J, Bonnet R: **Antibiotic Activity of Escherichia coli against Multiresistant Staphylococcus aureus.** Antimicrob Agents Chemother 2016, **60**(11):6986-6988.

18. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R et al: **Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences.** Nat Biotechnol 2013, **31**(9):814-821.

19. Reuter C, Alzheimer M, Walles H, Oelschlaeger TA: **An adherent mucus layer attenuates the genotoxic effect of colibactin.** Cell Microbiol 2018, **20**(2).

20. He Z, Gharaiheh RZ, Newsome RC, Pope JL, Dougherty MW, Tomkovich S, Pons B, Mirey G, Vignard J, Hendrixson DR et al: **Campylobacter jejuni promotes colorectal tumorigenesis through the action of cytolethal distending toxin.** Gut 2019, **68**(2):289-300.

21. Johnson JR, Oswald E, O'Bryan TT, Kuskowski MA, Spanjaard L: **Phylogenetic distribution of virulence-associated genes among Escherichia coli isolates**
associated with neonatal bacterial meningitis in the Netherlands. *J Infect Dis* 2002, **185**(6):774-784.

22. Martin P, Marcq I, Magistro G, Penary M, Garie C, Payros D, Boury M, Olier M, Nougayrede JP, Audebert M et al: *Interplay between siderophores and colibactin genotoxin biosynthetic pathways in Escherichia coli*. *PLoS Pathog* 2013, **9**(7):e1003437.

23. Datsenko KA, Wanner BL: *One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products*. *Proc Natl Acad Sci U S A* 2000, **97**(12):6640-6645.

24. Dubois D, Baron O, Cougnoux A, Delmas J, Pradel N, Boury M, Bouchon B, Bringer MA, Nougayrede JP, Oswald E et al: *ClbP is a prototype of a peptidase subgroup involved in biosynthesis of nonribosomal peptides*. *J Biol Chem* 2011, **286**(41):35562-35570.

25. Morin CE, Kaper JB: *Use of stabilized luciferase-expressing plasmids to examine in vivo-induced promoters in the Vibrio cholerae vaccine strain CVD 103-HgR*. *FEMS Immunol Med Microbiol* 2009, **57**(1):69-79.

26. Serino M, Luche E, Gres S, Baylac A, Berge M, Cenac C, Waget A, Klopp P, Iacovoni J, Klopp C et al: *Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota*. *Gut* 2012, **61**(4):543-553.

27. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C: *Metagenomic biomarker discovery and explanation*. *Genome Biol* 2011, **12**(6):R60.

28. Caraux G, Pinloche S: *PermutMatrix: a graphical environment to arrange gene expression profiles in optimal linear order*. *Bioinformatics* 2005, **21**(7):1280-1281.
Caecum microbiota of mice aged 15 days following colonization with genotoxic or non-genotoxic E. coli. A) Cladogram showing bacterial taxa significantly (P<0.01) higher in the group of mice of the same colour, according to the enterobacteria strain received at birth (the cladogram shows the taxonomic levels represented by rings with phyla at the inmost and genera/species at the outermost ring and each circle is a bacterial member within that level; the cladogram does not show the control group MG1655 meaning this group is not characterised by any higher bacterial taxon compared to the other groups); B) Principal Component Analysis (PCA) showing cluster of groups according to the caecum microbiota (following the colour code of the overall figure); P vs. control group (in blue); C) heat-map based on a Pearson distance and a complete linkage drawn with the PermutMatrix
software[28] showing multiple diversity indices and dot-plot representation of the Menhinick index on the right side; n=5.*P<0.05 vs. control group, Kruskal-Wallis test followed by a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli to correct for multiple comparisons by controlling the False Discovery Rate (<0.05).

Figure 2
Bacterial taxa reduction following colonization at day 15 with genotoxic or non-genotoxic E. coli. A)-F) phyla to species histogram representation. n=5. **P<0.01, ***P<0.001, ****P<0.0001, 2-ANOVA followed by a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli to correct for multiple comparisons by controlling the False Discovery Rate (<0.05).
Figure 3

Caecum microbiome of mice aged 15 days following colonization with genotoxic or non-genotoxic E. coli. A) Cladogram showing bacterial functions significantly (P<0.01) higher in the group of mice of the same colour, according to the enterobacteria strain received at birth; B) list of the bacterial functions and the Linear Discriminant Analysis (LDA) score (P<0.01) based on which the cladogram in (A) was built. n=5.
Caecum microbiota of mice aged 35 days following colonization with genotoxic or non-genotoxic E. coli. A) Cladogram showing bacterial taxa significantly (P<0.01) higher in the group of mice pups of the same colour, according to the enterobacteria strain received at birth; B) PCA showing cluster of groups according to the caecum microbiota (following the colour code of the overall figure); P vs. genotoxic group (in red); C) heat-map based on a Pearson distance and a complete linkage drawn with the PermutMatrix software showing multiple diversity indices and dot-plot representation of the Simpson, Chao-1, Margalef and Fisher-alpha indices on the right side; n=5.*P<0.05, **P<0.01 vs. control group, Kruskal-Wallis test followed by a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli to correct for multiple comparisons by controlling the False Discovery Rate (<0.05), $P<0.05, $$P<0.01 between SP15clb- and SP15clb+ groups, Mann-Whitney test.
Figure 5

Bacterial taxa reduction following colonization at day 35 with genotoxic or non-genotoxic E. coli. A)-F) phyla to species histogram representation. n=5. ****P<0.0001, 2-ANOVA followed by a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli to correct for multiple comparisons by controlling the False Discovery Rate (<0.05).
Caecum microbiome of mice aged 35 days following colonization with genotoxic or non-genotoxic E. coli. A) Cladogram showing bacterial functions significantly (P<0.01) higher in the group of mice pups of the same colour, according to the enterobacteria strain received at birth B) list of the bacterial functions and the LDA score (P<0.01) based on which the cladogram in (A) was built. n=5.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

Supplementary Figures Legends.docx
Tronnet et al. Fig.S4.JPG
Tronnet et al. Fig.S5.JPG
Tronnet et al. Fig.S1.JPG
Tronnet et al. Fig.S2.JPG
Tronnet et al. Fig.S3.JPG