Transmission and persistence of multidrug resistance in the human infant gut microbiota By Ravi Anuradha[1,*], Ekaterina Avershina[1,*], L'Abée-Lund Trine[2], Jane Luvigsen[1], Torbjørn[3] Øien, Ola Storrø[3], Roar Johnsen[3] and Knut Rudi[1]

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Abstract:

The development and spread of multidrug antibiotic resistance (AR) represent a major public health threat. The reservoirs and mechanisms for multidrug resistance development are largely unknown. Here we addressed the transmission and persistence of class I integrons (multidrug resistance assembly unit) in the gut microbiota from a large cohort of mothers and their children. We used a combination of quantitative PCR and metagenome sequencing. We found out that, even with the constant changes in the infant gut microbiota integrons showed a high persistence. An integron-containing plasmid belonged to incompatibility group I and held resistance genes to gentamycin and streptomycin was identified. We propose that that multidrug resistance can persist in the human commensal gut microbiota through mobile genetic elements.

Introduction:

The increased antibiotic usage has caused the spread of antibiotic resistance (AR) genes representing a major threat to global health – particularly through the development of multidrug resistance (1). Important vectors for the transfer and assembly of AR genes are integrons.

Integrons are assembly platforms for integration and expression of mobile promoterless genes called as gene cassettes (2). The integrons are mostly found on mobile genetic elements like plasmids or transposons. There are five classes of integrons, but AR genes are mostly linked to class I integrons.

The human intestinal microbiota is a complex and diverse environment(3). It plays beneficial roles in immune development and resistance to pathogens. Recent reports have shown increased horizontal gene transfer rate in the gut microbiota compared to any other environment(4). What is still unknown, however, is the role of the gut microbiota in the development of multidrug resistance. Our aim was therefore to use a combination of 16s rRNA gene- , metagenome sequencing and quantitative PCR to study the distribution and persistence patterns of integrons as AR gene assembly units and trace the elements longitudinally in a large cohort of mothers and their children.

Materials and methods:

IMPACT (Immunology and Microbiology in Prevention of Allergy among Children in Trondheim) is a non-randomized longitudinal study cohort. The study consists of 720

pregnant women and their children of up to two years of age. The fecal samples were collected during the first/second (7-20 weeks) trimester and the third (32-40 weeks) trimester from the mothers, and at 10 days, 4 months, 1 and 2 year of the child.

Initially, the presence/absence of integron (*int*1 gene(5)) in the samples was screened and the abundance was calculated relatively by the 16s rRNA gene quantification. High Resolution Melting (HRM) curve analysis and Sanger sequencing was used for the verification of the *int*1 gene.

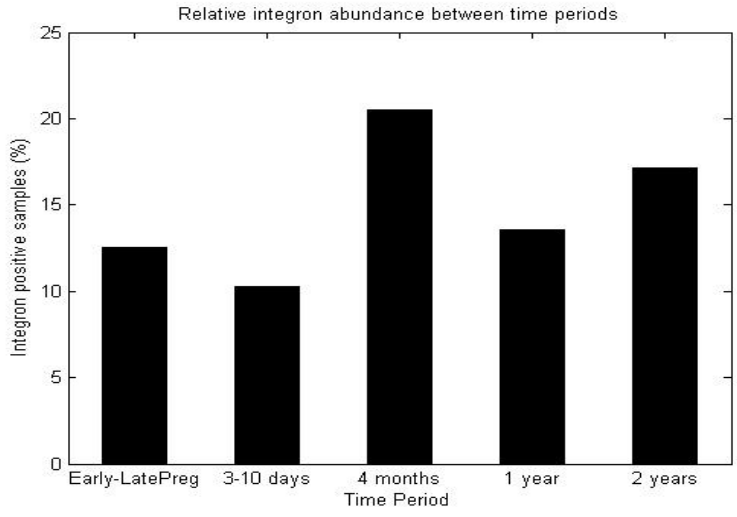
Shot-gun metagenome sequencing for selected samples was performed by Illumina Nextera XT kit where the genomic DNA was randomly fragmented; tagged; amplified according to the instructions provided by Illumina.

Metagenome data mapping and assembly was done in Geneious pipeline and CLC main workbench. The taxonomic and phylogenetic information of the samples were annotated by MG-RAST analyzer.

Results:

The presence/absence of the integrons by quantitative PCR showed that 99 of 681 samples showed the presence of integrons. While checking the prevalence and persistence patterns of the integrons, they were mostly prevalent at 4 months of the child and gradually decreased with age (Figure 1a) and persistent from 10 days to 4 months, in addition to between the mother and child (Figure 1b).

a)



b)

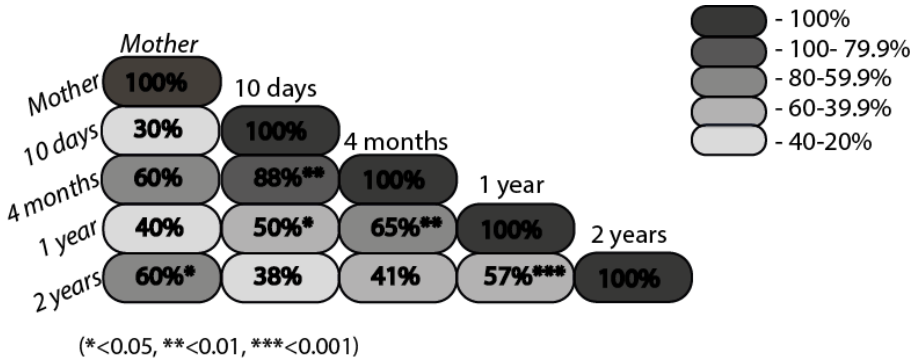


Figure 1: Presence and persistence of integron-positive samples between time periods. **a)** Relative population of integron positive samples in the dataset. **b)** Persistence of integrons. *indicates the significant levels

Fifteen samples that showed highest relative *int*1 abundance and contained microbiota profile information (taxonomy profile by 16s rRNA sequencing) were selected for shot-gun metagenome sequencing. In average, 837048 reads were obtained from each sample and the reads were submitted to MG-RAST analyzer. All the samples showed the presence of integrons or integron- related genes. The samples showed varying number of hits to integrons in PATRIC database (Figure 2) which is shown as the abundance of integrons. The number of hits was highest at 10 days and 4 months of the child.

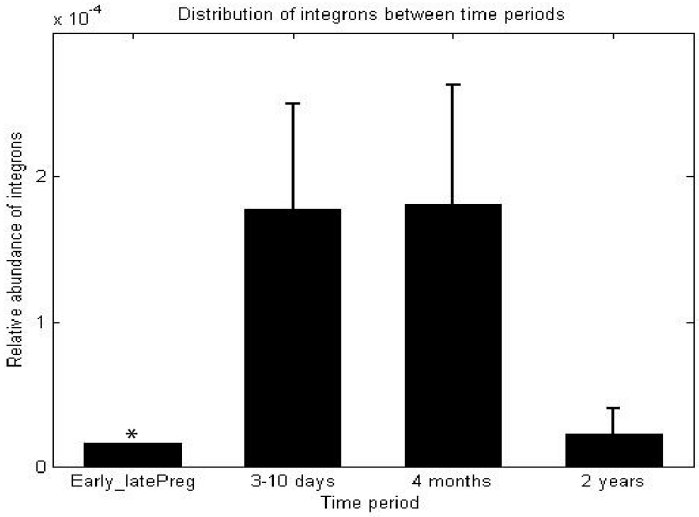


Figure 2: Distribution of integron hits from PATRIC database at each time point from MG-RAST analysis. *Only one sample from early_latePreg was taken.

The reads that showed *int*1 homology were extracted and BLAST searched against the NCBI database. Two candidate plasmids pCFSAN002069_01, GenBank CP005389; and pECN580, GenBank KF914891 were the most prevalent hits from two samples taken from the same child at 10 days and 4months. The reads were mapped back to the complete plasmid sequences. pCFSAN002069_01 showed the highest coverage (20x and 34x) in both 10 days and 4 months.

De novo assembling of the reads into contigs has revealed one contig (2277bp) mapping the pCFSAN002069_01 plasmid containing the *int*1 gene. The integron-containing plasmid belonged to the incompatibility group 1 containing *dfr*17 and *aad*7 genes. The genes encode resistance to streptomycin and gentamycin. The plasmid revealed homology to plasmids from *Escherichia coli*, *Salmonella enterica* and *Shigella flexneri*.

Discussion:

The human gut microbiota goes through various perturbations during the first year of life until it reached the 'adult-like state' with a more or less stable microbiota. In spite of these constant changes in the microbiota, the integron seems to survive by being prevalent and persistent at all ages. Interestingly, the 16s rRNA profiling of the microbiota does not show any bacterial species linking to the persistence patterns of the integron (data not shown). This could mean that integrons are under horizontal transfer within the microbiota.

Moore *et al* emphasizes the presence of cryptic, not distinguishable organisms as potentially important antibiotic reservoirs in pediatric fecal microbiota(6). Here we show the potential role of integrons in assembly and persistence of multidrug AR resistance in the gut microbiota.

References:

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