Antibiotic Resistance and Fitness of *Escherichia coli* in the Infantile Commensal Microbiota

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborg universitet, kommer att offentligen försvaras I föreläsningsalen, våning 3, Guldhedsgatan 10A, Göteborg

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Av Nahid karami

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Ahandlingen baseras på följande delarbeten:

I. Nahid Karami, Ingegerd Adlerberth and Agnes Wold. Virulence traits, phylogenetic groups and antibiotic resistance in intestinal and urinary *Escherichia coli* from Swedish infants. In manuscript.

II. Nahid Karami, Ingegerd Adlerberth and Agnes Wold. Tetracycline resistance in *Escherichia coli* and persistence in the infantile colonic microbiota.
Antimicrobial Agents and Chemotherapy, Jan. 2006, P. 156-161.

III. Nahid Karami, Charles Hannoun, Ingegerd Adlerberth and Agnes Wold. Impact of β-lactamase gene carriage on *in vivo* fitness of *Escherichia coli* in the infantile colonic microbiota. Submitted for publication.

IV. Nahid Karami, Anna Martner, Virve I. Enne, Svante Swerkersson, Ingegerd Adlerberth and Agnes Wold. Transfer of ampicillin resistance genes followed by mutational changes during natural colonization by *Escherichia coli* in the infantile bowel. Submitted for publication.

V. Nahid Karami, Ingegerd Adlerberth and Agnes Wold. Clonal group A (CGA) *Escherichia coli* involved in transfer of antibiotic resistance genes in the intestinal microbiota. In manuscript.

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Microbial resistance to antibiotics is a growing problem worldwide. Resistance develops not only in microbes which are the targets of the antibiotic treatment, but also in those belonging to the normal microbiota of the treated host. Little is known on the ecological consequences of antibiotic resistance in commensal bacteria. *Escherichia coli* belongs to the normal intestinal microbiota but may also cause urinary tract infection (UTI) or septicaemia if spread from the bowel. The present thesis studies the prevalence and stability of antibiotic resistance among *E. coli* colonizing the gut of healthy infants and its impact on *in vivo* fitness. We also examine evidence for transmission of resistance genes between *E. coli* strains during simultaneous presence in the bowel microbiota.

E. coli was isolated from faecal samples obtained at regular intervals from 128 Swedish infants. For comparison, a collection of 205 urinary *E. coli* isolates from 205 infants 0-2 years of age was examined. Individual *E. coli* strains were identified by RAPD, phylogenetic grouping and virulence gene carriage. Their phenotypic resistance to 14 clinically relevant antibiotics was examined and genes encoding resistance to tetracycline and ampicillin were identified.

Twelve percent of commensal *E. coli* strains, but 40% of urinary isolates were resistant, most commonly to tetracycline, ampicillin or trimethoprim. Both tetracycline and ampicillin resistant *E. coli* strains were equally capable of persisting in the intestinal microbiota as susceptible strains and their resistance genes were mostly kept during the entire colonization period. Most resistant strains established in infants who had never received any antibiotics. Tetracycline resistant *E. coli* strains mostly belonged to phylogenetic group A and ampicillin resistant *E. coli* strains to phylogenetic group D. The tetracycline resistance gene *tet*A was associated with the *iutA* virulence gene, encoding aerobactin, while *tet*B and *bla*TEM encoding ß-lactamases was associated with *papC*, encoding P fimbriae.

In two cases, we could demonstrate transmission of plasmids carrying *bla*TEM genes from ampicillin resistant *E. coli* strains to initially sensitive *E. coli* strains colonizing simultaneously in the infant's bowel microbiota. Both donor strains belonged to phylogenetic group D. In the first case, the infant was treated with ampicillin, which enhanced the population counts of the donor strain and provided a selective pressure promoting the survival of the transconjugant strain. Further, mutation of the *bla*TEM promoter gene was observed in the donor strain, leading to a more effective expression of the *bla*TEM gene. In the second case, transfer of a plasmid conferring resistance to ampicillin, streptomycin and sulphonamide was demonstrated in an infant who was not treated with antibiotics. The donor strain belonged to the globally spread uropathogenic CGA clone.

Our study is the first to examine the stability and fitness costs of resistance gene carriage in commensal bacteria in human hosts under natural conditions. Our results indicate that resistance genes are prone to spread among strains in the intestinal microbiota and that strains belonging to group D may be especially apt to participate in such gene transfer. Furthermore, the fitness cost of resistance gene carriage appears to be small and may be compensated for by simultaneous carriage of genes encoding colonization-promoting factors, such as P fimbriae and aerobactin.

Key words; ampicillin, tetracycline, *in vivo* fitness, persistence, gene transfer, intestinal, *bla*TEM *Escherichia coli*.

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