# Phenotyping and genotyping of non–*Escherichia coli* Enterobacteriaceae isolated from the gut microbiota of healthy subjects.

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Strains of *Escherichia coli* in the gut microbiota of healthy people have been thoroughly investigated, as they can be involved in opportunistic morbidity. Conversely, exploration of non–*E. coli* Enterobacteriaceae (NECE) from healthy subjects is still at its infancy, and deeper *in vivo* studies are necessary to understand the capability to became virulent induce infections. NECE strains were isolated from the feces of 20 healthy adults. Bacterial isolates were obtained from HiCrome Coliform agar plates and assayed with Kovac's test to differentiate *E. coli* from NECE. *E. coli* outnumbered NECE, the latter being on average < 7.5% of the isolates. A total of 357 NECE isolates were clustered by RAPD-PCR and ERIC-PCR into 32 different biotypes. They were taxonomically classified by partial sequencing of 16S rRNA gene and by MALDI-TOF. Fifteen isolates were attributed to *Klebsiella* (11 *K. pneumoniae* and 4 *K. oxytoca*), eight to *Enterobacter* (6 *E. cloacae*, 1 *E. aerogenes*, and 1 *E.r kobei*), two to *Cronobacter* sp., four to Citrobacter (3 *C.r freundii* and 1 *C. amalonaticus*), and one to each *Hafnia alvei*, *Morganella morganii*, and *Serratia liquefaciens*. The biotypes were screened with a PCR assay for the presence 19 virulence genes—13 from *K. pneumoniae* (*allS*, *entB*, *irp-1*, *irp-2*, *K2*, *kfu*, *kpn*, *magA*, *mrkD*, *rmpA*, *ybtS*, and *ycfM*) and 6 of *E. coli* (*fimH-1*, *fyuA*, *iroN*, *iutA*, and *traT*,)—which have cognates in the species herein identified. The vast majority of *K. pneumoniae* isolates (6–10) harbored *kpn*, *entB*, *irp-2*, *mrkD*, and ycfM (encoding adhesins and siderophores), while only a minority (1–2) were positive to *allS*, *kfu*, *irp-1*, *ybtS*, *fyuA*, *iutA*. *K. pneumoniae* harbored only *kpn* and *ybtS*. All the *E. cloacae* and one of the *Citrobacter* sp. harbored *irp-2*. *entB* and *mrkB* were the sole other virulence genes, observed in a minority of biotypes (1–2) within *Enterobacter*. Most of NECE strains (27) showed strong biofilm production in synthetic medium, including all *Cronobacter* and *K. oxytoca* and generally *Citrobacter*, *Enterobacter*, and *K. pneumoniae.* Congo red and calcofluor white staining were utilized to screen the strains for curli and extracellular cellulose structures, respectively. All the isolates belonging to *Citrobacter*, *E. cloacae*, *H. alvei*, and one to *K. oxytoca* produced both cellulose structures and curli. The isolates of *Cronobacter* sp. produced curli but not cellulose. *K. pneumoniae* did not form curli, while few strains produced cellulose. *E. kobei* and *M. morganii* formed curli, while *S. liquefaciens* cellulose structures. Susceptibility to eight antibiotics (amikacin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole) was assayed. NECE strains resulted generally sensitive to all the tested antibiotics. Resistance to amoxicillin-clavulanic acid was observed only for all the strains of *Citrobacter*, *Enterobacter*, *Hafnia*, *Morganella*, and *Serratia.* As a whole, enterobacteriaceae from healthy subjects are still sensitive to most of the antibiotics, confirming that the problem of antibiotic resistance is restricted to frequent and inappropriate use the antibiotics, and to the hospital setting where antibiotic pressure is highest.