

TITLE: Diversity of virulence-encoding genes in ancient *Raoultella* spp. causing intestinal infections in humans

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Raoultella spp. was dismembered from the genus *Klebsiella* in 2001, and now it is composed by *R. electrica*, *R. ornithinolytica*, *R. planticola*, and *R. terrigena*. Although *Raoultella* spp. is routinely recovered from environmental sources, recent reports have evidenced the emergence of infections caused by such group of microorganisms. Herein, we aimed to analyze a collection of *Raoultella*, which were misidentified as *Klebsiella* spp. strains, and to characterize their pathogenic potential. We analyzed 67 *Klebsiella* spp. strains recovered from intestinal and extraintestinal infections during the 80s-90s in the city of São Paulo, Brazil. The identification at genus and species levels was confirmed by MALDI-TOF MS and, for those isolates identified as *Raoultella* spp., a total of 24 distinct virulence-encoding genes frequently found in *Klebsiella* spp. strains were screened by PCR. The ability to form biofilm on abiotic surface, the hypermucoviscosity phenotype, hemolytic activity in blood agar, and adherence on HeLa and polarized Caco-2 cells (6 h) were also evaluated. The antimicrobial susceptibility profile was determined against 14 antimicrobials by disk diffusion method according to EUCAST guidelines. A total of 6/67 (8.9%) strains obtained from fecal samples of patients with intestinal infections in 1987, where no other enteropathogen was found, were identified as belonging to the genus *Raoultella*. Among them, three isolates were identified as *R. ornithinolytica* followed by *R. planticola* (n=2), and *Raoultella* spp. (n=1). The virulence-encoding genes *entB*, *ybtS*, *iutA*, *aeroI*, and *mrkD* were present in 100% of the strains. In addition, the *kpn* (83.3%), *aero2* (83.3%), *irp2* (50%), *fyuA* (50%), *pagO* (50%), *fimH* (50%), *shiF* (33.3%), *traT* (33.3%), and *kfu* (33.3%) genes were also detected. The number of positive virulence determinants varied from seven to nine. Only one strain (16.7%) formed a strong biofilm. All strains were negative for hypermucoviscosity phenotype and hemolytic activity but were able to adhere on HeLa and Caco-2 cells at different intensities. Finally, the *Raoultella* spp. strains were susceptible to most of the antimicrobials tested, except for five strains (83.3%) that showed resistance to fosfomicin. Our results identified *Raoultella* spp. strains in feces of diarrheagenic patients, which carried many virulence genes, suggesting the potential of *Raoultella* species to cause diarrhea in humans.

Keywords: virulence, *Raoultella* spp., Gram-negative bacilli, Enterobacteriales, diarrhea.

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