

23309 | Colonization and diversity of *Klebsiella pneumoniae* species complex (KpSC)
in the healthy gut: The potential role of the food chain and lifestyle factors

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Background & Aim: *Klebsiella pneumoniae* species complex (KpSC) includes *K. pneumoniae* (Kp), a gut pathobiont with an unclear role in healthy humans (HH) and a debated foodborne origin [1,2]. We studied KpSC fecal carriage, diversity, and risk factors in HH. **Methods:** Fecal samples from 51 northern Portuguese HH (29-women; 18-85/~45-years; 16-cities; February-July/2022) were enriched and plated on SCAI±colistin. KpSC were identified by PCR+sequencing (*wzi*-K-type), tested for standard antimicrobial susceptibility (EUCAST/CLSI), *silA* gene PCR-screening for copper decreased susceptibility and analyzed via WGS/PathogenWatch. An ethics-approved survey was completed (epidemiological/clinical/ food/antibiotics/other factors). Binomial test/ χ^2 /logistic regression were used (SPSS v28). **Results:** Eighteen samples (35%) carried KpSC [66 isolates/59 Kp; 1-9 per sample]. Seventeen K-types (K13/38/60 shared) were identified, most individuals with 1 K-type (n=14) ($p<0.05$). Two samples carried tetracycline±ciprofloxacin-resistant isolates, with all others antibiotic-susceptible, including colistin (MIC<1mg/L). Isolates *silA*⁺ were found in 13 (70%) samples (55 isolates; 10 K-types). Gastrointestinal symptoms and white meat consumption were associated with KpSC-*silA*⁺ carriage ($p<0.05$). *Escherichia coli* and *Enterococcus* co-occurred in 88% and 98% of samples, respectively. Higher *Enterococcus* species diversity was linked to KpSC carriage (OR=2.06, 95%CI: 1.07–3.94). No significant associations were found with other lifestyle factors ($p>0.05$). **Conclusions:** This study reveals diverse gut-colonizing KpSC strains coexisting with *E.coli* and *Enterococcus*, highlighting the need to investigate lifestyle factors in KpSC carriage. The high occurrence of *silA*⁺ strains, linked to food-animal environments, and the absence of colistin-resistant strains during colistin withdrawal in Portugal [2] suggest a food-chain role in KpSC acquisition [2,3]. Continuous surveillance is crucial for public health under a One-Health approach.

Keywords: Human gut, *Klebsiella pneumoniae* species complex, lifestyle factors, food-chain, One-Health.

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References:

- [1] Lepuschitz S, Hauser K, Schriebl A, et al. Fecal *Klebsiella pneumoniae* Carriage Is Intermittent and of High Clonal Diversity. *Frontiers in Microbiology*. 2020;11:581081. Published 2020 Nov 24. doi:10.3389/fmicb.2020.581081
- [2] Mourão J, Magalhães M, Ribeiro-Almeida M, et al. Decoding *Klebsiella pneumoniae* in poultry chain: unveiling genetic landscape, antibiotic resistance, and biocide tolerance in non-clinical reservoirs. *Frontiers in Microbiology*. 2024;15:1365011. doi:10.3389/fmicb.2024.1365011
- [3] Ribeiro S, Mourão J, Novais Â, Campos J, Peixe L, Antunes P. From farm to fork: Colistin voluntary withdrawal in Portuguese farms reflected in decreasing occurrence of mcr-1-carrying Enterobacteriaceae from chicken meat. *Environmental Microbiology*. 2021;23(12):7563-7577. doi:10.1111/1462-2920.15689