

## HIGH IMPACT OF THE MEAT PROCESSING ENVIRONMENT ON THE PREVALENCE OF KLEBSIELLA SPP. IN BELGIUM.

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### INTRODUCTION

The opportunistic bacterium *Klebsiella pneumoniae* is present in many niches, such as in animals and food, and is a known trafficker of antibiotic resistance genes. In this study, we investigated the prevalence and antibiotic resistance of *Klebsiella* spp. in primary production and distribution environments within food production in Belgium. We aimed to gain insight into the possible sources of contamination with *Klebsiella* spp. and its transmission routes in food-producing animals and their products.

### METHODOLOGY

In total, 1508 samples of the Belgian national antimicrobial resistance monitoring plan for zoonotic bacteria - including meat at distribution ( $n = 905/1508$ , 60.01 %), raw milk ( $n = 16/1508$ , 1.06 %), and faeces samples at primary production ( $n = 587/1508$ , 38.93 %) from pigs, poultry and cattle - were analysed. In brief, 1 g of faeces was immersed in 9 ml of buffered peptone water (BPW) and incubated at  $37 \pm 1^\circ\text{C}$  for  $20 \pm 2$  h. A total of 10  $\mu\text{l}$  was streaked onto MacConkey agar (MC). Raw milk was analysed by streaking 10  $\mu\text{l}$  directly onto MC and incubating at  $37 \pm 1^\circ\text{C}$  for  $20 \pm 2$  h. Meat samples were processed using a 25 g sample immersed in 225 ml BPW. This was incubated at  $37 \pm 1^\circ\text{C}$  for  $20 \pm 2$  h and a total of 10  $\mu\text{l}$  was streaked onto MC. Based on morphology, one to three typical colonies were transferred onto nutrient agar for identification by MALDI-TOF MS. Confirmed *Klebsiella* spp. and *Raoultella* spp. were screened for resistance using MC+cefotaxime (MC+CTX 1 mg/l) and CarbaSMART to screen for ESBL and carbapenem resistance, respectively. Minimum inhibitory concentration (MIC) analysis was performed by broth microdilution using EUVSEC 2 and 3 following the EUCAST guidelines.

### RESULTS

A positivity rate of 25.19 % (228/905) of *Klebsiella* spp. and/or *Raoultella* spp. was observed for the meat samples, for the primary production samples, this rate was 2.15 % (13/603). From 241 isolates, only three *K. pneumoniae* isolates were able to grow on MC+CTX and no growth was observed on CarbaSMART. MIC analysis was performed on all isolates originating from primary production. Only two isolates showed resistance to tetracycline, no other resistances were observed. Among the three ESBL strains isolated from the meat samples, one isolate showed additional carbapenem resistance with an MIC value of 2 mg/L

for ertapenem and 0.5 mg/L for meropenem. Whole genome sequencing for the complete characterisation of this strain is in progress.

## DISCUSSION

In this study, 25 % of the samples coming from distribution were positive for *Klebsiella* spp. and/or *Raoultella* spp. while their prevalence in primary production was low (2.15 %). We hypothesise that the meat processing environment is linked to the high number of positive results for the meat samples, however further analysis is necessary. The presence of a carbapenem-resistant *Klebsiella pneumoniae* isolate in chicken meat is a worrisome finding. More research should be carried out to track down the transmission route of *Klebsiella* spp. and *Raoultella* spp. along the farm to fork chain.