Multidrug-Resistant *Klebsiella pneumoniae* in Native Portuguese Cattle Breeds: A Public Health Concern

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INTRODUCTION & AIM

- The emergence of antibiotic-resistant bacteria, especially multidrug-resistant strains, has reduced the effectiveness of treatments [1]. Livestock act as reservoirs, spreading resistant bacteria through the food chain and the environment [2]. *Klebsiella pneumoniae* is a major concern due to its role in severe human infections, veterinary relevance, and resistance to multiple drugs [3–5].

- This study aimed to phenotypically and genotypically characterize *Klebsiella* spp. isolated from fecal samples of four cattle breeds: Holstein-Friesian and three native Portuguese breeds - Barrosã, Cachena, and Minhota (Figure 1, 2), in Northern Portugal.

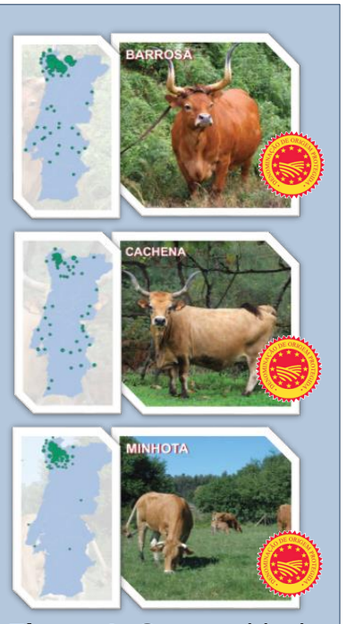

Figure 1. Geographical distribution of native cattle breeds [6].

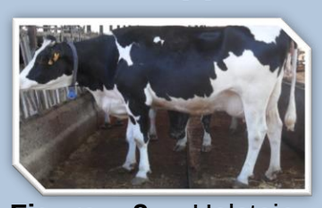

Figure 2. Holstein-Friesian cattle breed.

METHODS

- A total of 640 fecal samples were collected from 40 farms, pooled by age group (8 calves and 8 cows per farm) [7] and inoculated on selective and differential agar (Figure 3).

- A representative subset of *Klebsiella* spp. isolates (n=43 out of 63) was selected for antibiotic susceptibility testing and ESBL activity assessment (CLSI guidelines), as well as PCR assays for species identification and detection of resistance genes [4] (Figure 4).

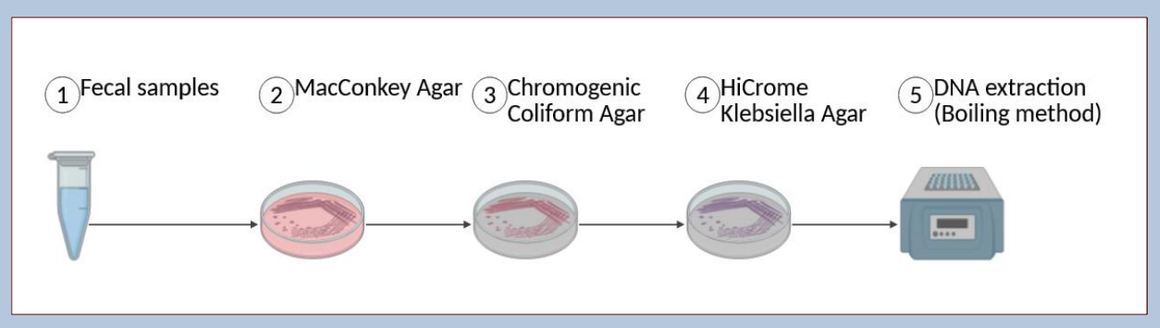

Figure 3. Cultivation and selection of presumptive *Klebsiella* spp. isolates.

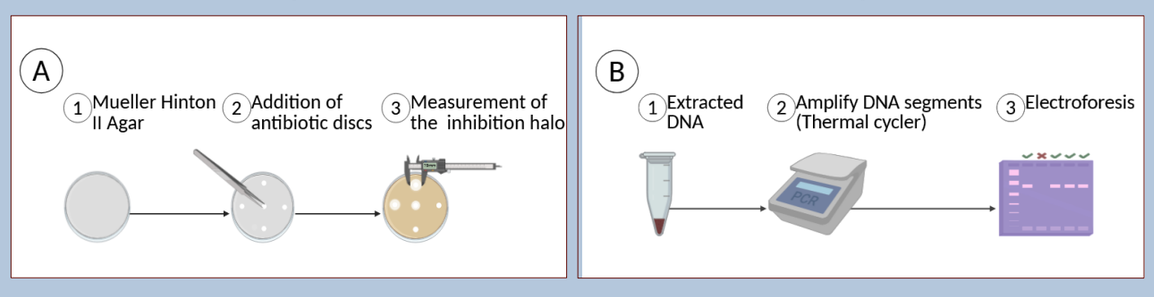

Figure 4. A. Antimicrobial susceptibility and ESBL activity tests (n=43), **B.** Identification of *Klebsiella pneumoniae* and genotypic antibiotic resistance characterization by PCR assay (n=42).

RESULTS & DISCUSSION

- All isolates were identified as *K. pneumoniae* and showed resistance to at least one antibiotic; 100% were intrinsically resistant to ampicillin. Multidrug resistance was observed in 14% of isolates, all from Holstein-Friesian cattle (Figure 5 and 6).

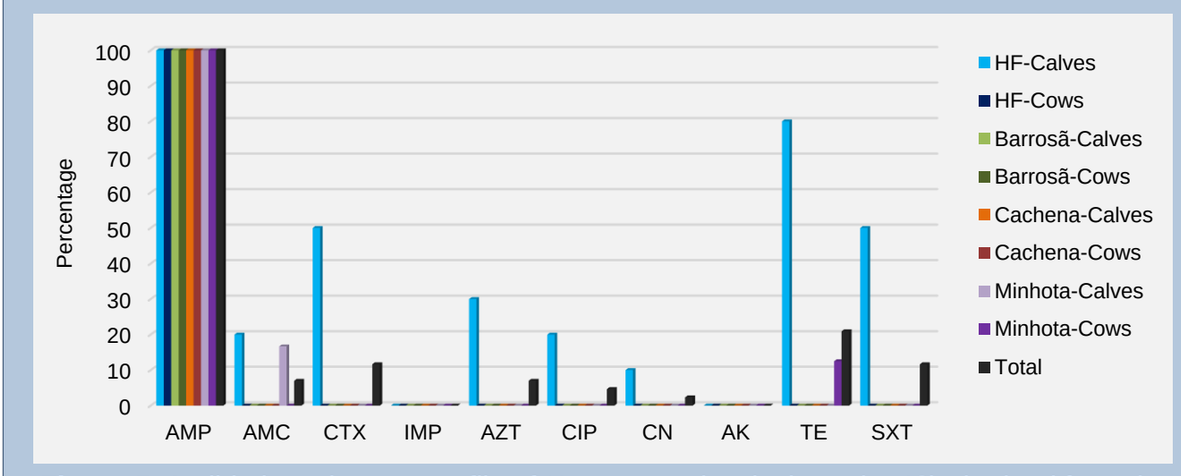

Figure 5. Antibiotic resistance profile of *K. pneumoniae* isolates (n=43) obtained from the four cattle breeds fecal samples.
AMP: ampicillin; AMC: amoxicillin + clavulanic acid; CTX: cefotaxime; IPM: imipenem; AZT: aztreonam; CIP: ciprofloxacin; CN: gentamicin; AK: amikacin; TE: tetracycline; SXT: trimethoprim+sulfamethoxazole; HF: Holstein-Friesian.

- ESBL activity was detected in 21% of the 43 *K. pneumoniae* isolates from Holstein-Friesian and Minhota cattle (Figure 6).

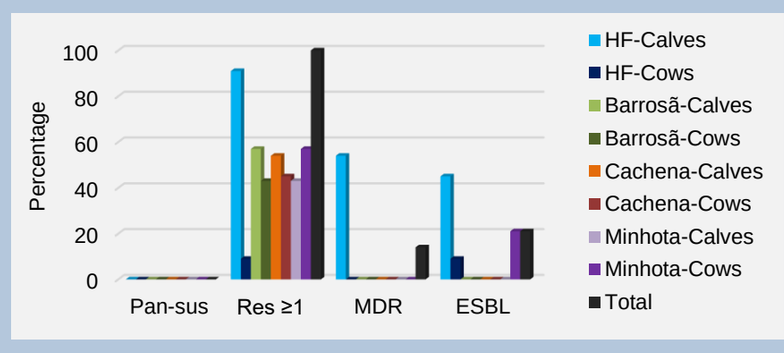

Figure 6. Characterization of antimicrobial resistance and distribution of ESBL activity among isolates from diferent cattle breeds (n=43).
Pan-sus: pan-susceptibility; Res ≥1: resistance to ≥1 antibiotic; MDR: multidrug-resistant phenotype.

- The bla_{SHV} gene, indicator of intrinsic ampicillin resistance, was detected in 67% of isolates, followed by *sul*2 (43%), *aac*(3′)-IV (43%), bla_{TEM} (33%), bla_{CTX-M} (14%), *tet*B, and *aac*(6′)-Ib-cr (10% each), with higher prevalence in calves (Figura 7).

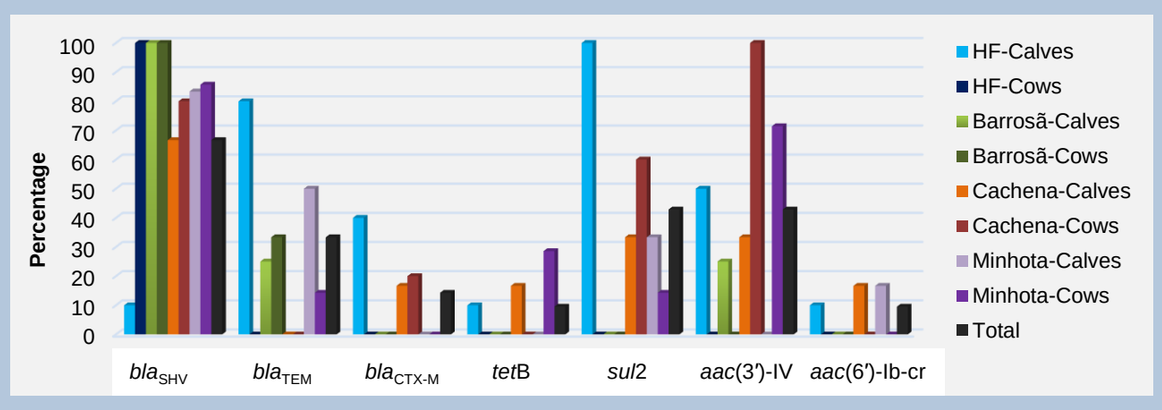

Figure 7. Genotypic characterization of antibiotic-resistant *K. pneumoniae* isolates (n=42).

CONCLUSION

- This work underscores, for the first time, the presence of antibiotic-resistant *K. pneumoniae* in native Portuguese cattle breeds, highlighting potential risks to food safety and public health. However, these isolates showed greater antibiotic susceptibility compared to those from Holstein-Friesian dairy cattle. This may reflect more limited antibiotic exposure in native breeds, potentially reducing the risk of spreading multidrug-resistant strains.

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