EMERGENCE OF MAJOR INTERNATIONAL HIGH-RISK CLONES IN COMPANION ANIMALS WITH URINARY TRACT INFECTION: 16 YEARS RETROSPECTIVE STUDY IN PORTUGAL

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Urinary tract infections caused by multidrug resistant bacteria are being increasingly reported in companion animals[1]. Antimicrobial choice should account for the most likely causative agent and local susceptibility patterns[2]. Thus, updated information on antimicrobial susceptibility is crucial. Furthermore, since companion animals with UTI are generally treated at home, their possible role in the spread of important bacterial clones to humans is a major concern.

This retrospective study first goal was to determine antimicrobial resistance temporal trends over 16 years in bacteria isolated from companion animals with UTI in Portugal. Also, it aimed to evaluate the clonal lineages of bacteria harboring critical antimicrobial resistance mechanisms.

Susceptibility testing was conducted by disk diffusion method in 966 bacteria isolated from companion animal positive urine cultures (1999-2014). Detection of resistance mechanism was conducted by PCR for: third generation cephalosporin resistance (3GC) ESBL/pAMPc in Gram negatives, *mec*A in methicillin resistant staphylococci (MRS), *aac(6')-Ieaph(2'')-Ia* and *aph*(2'')-1d in high level gentamicin resistant (HLGR) enterococci. The clonal lineages of resistant bacteria were determined by MLST. Staphylococci were also typed by SCC*mec*. *E. coli* ST131 O25b serotyping was conducted by PCR. Statistical analysis of antimicrobial resistance temporal trends were determined by logistic regression model of SAS.

E. coli (43.4%, n=419/966) was the most frequently isolated bacteria followed by Proteus mirabilis (16.0%, n=155/966), *Staphylococcus* spp. (13.2%, n=128/966) and *Enterococcus* spp. (6.8%, n=66/966). Gram negative resistance to amoxicillin/clavulanate, 3GC, fluoroquinolones and trimethoprim/sulphamethoxazole had a statistically significant increase over time ($p<0.0001$, $p<0.0001$, $p<0.0001$ and $P=0.0095$, respectively). An increasing temporal trend in MDR Gram negative bacteria was also detected ($p<0.0001$). 3GC-resistance was mainly due to the presence of *bla*CTX-M-15 in Klebsiella, *bla*CMY-2 in *P. mirabilis* and to *bla*CTX-M-15, -32 and CMY-2 in *E. coli*. These resistance mechanisms are known to be highly important in human medicine. 3GC-resistant *E. coli* lineages showed a marked temporal variation, with the emergence of two major uropathogenic lineages associated with UTI in humans[3-4], namely: O25b:H4-B2-ST131 (14.8%, n=4/27) first detected in 2004 and ST648 (37.0%, n=10/27) first detected in 2010. All *Klebsiella pneumoniae* 3GC-resistant were multi-drug resistant and also belonged mainly to the international MDR zoonotic *K. pneumoniae* lineage ST15 (n=10/13).
MRS due to the presence of *mec*A gene was detected in 8.6% (n=11/128) *Staphylococcus* spp.. MR*S. pseudintermedius* and *S. epidermidis* belonged to ST71-II-III, ST196-V and to ST2-nt, ST20-nt, ST23-IV, ST35-nt, respectively. One ST5-VI and one ST105-II MR*S. aureus* were also isolated.
HLGR was found in *Enterococcus faecalis* (13.2%, n=7/53) harboring *aac(6')-Ieaph(2'')-Ia*, and *Enterococcus faecium* (n=2/9) harboring *aac(6')-Ieaph(2'')-Ia* or *aph*(2'')-1d. Almost all *E. faecium* were ampicillin resistant (n=8/9). Two HLGR high risk *E. faecalis* lineages, ST6 (CC2) and ST16, were found. *E. faecalis* clonal-complex 2 (CC2) is particularly important since it is a human hospital-adapted complex. Two resistant *E. faecium* were also found to belong to human hospital-adapted clonal-complex, namely ST19 (CC17) and ST137 (CC17).

This study shows increasing temporal trends in antimicrobial resistance in uropathogens from companion animals. The clear emergence of major international high-risk lineages harboring critical resistance mechanisms in companion animals should not be neglected and raises great public-health concerns. Measures should be taken during active UTI in companion animals to minimize the spread of such bacteria.

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