Virulence factors and antimicrobial resistance of *Escherichia coli* isolated from canine pyometra and its relationship to biofilm formation

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Our laboratory recently demonstrated that a majority of *Escherichia. coli* (*E. coli*) isolates associated with canine pyometra produce biofilm both in vivo and in vitro. Biofilm, an extracellular matrix of polysaccharides, protects a microorganism from the host immune system and antimicrobial agents. Moreover, multiple virulence factors and antimicrobial resistance genes have been identified in this microorganism which play a role in its pathogenesis. We hypothesized that *E. coli* isolates capable of biofilm production display increased resistance to antimicrobials and carry more resistance genes and virulence factors. The objectives were to compare virulence factors and susceptibility patterns to antimicrobials between strains of *E. coli* that are biofilm producers versus non producers. Fourteen strains of *E. coli* isolated from clinical cases of canine pyometra were utilized. Of the 14 strains, 11 strains were biofilm forming and 3 strains were non biofilm forming. Bacteria were cultured on MacConkey and blood agar for 24 hours. Antimicrobial susceptibility testing to commonly used antibiotics and in vitro biofilm production testing were performed at The Ohio State University Veterinary Medical Center Microbiology Laboratory. Whole genome sequencing of isolates was performed at the Ohio Department of Agriculture, Animal Disease and Diagnostic Laboratory to determine presence of known virulence factors and antimicrobial resistance genes. In vitro antimicrobial sensitivity, presence of antimicrobial resistance genes, and virulence factors were compared between groups by Fisher's Exact test, using StatPlus software. Significance was set at $p < 0.05$. Biofilm producers were resistant to more antimicrobials (9/19) than nonbiofilm producers (2/19). All *E. coli* samples (14/14) were resistant to amoxicillin/clavulanic acid and ampicillin, common antibiotics used for treatment of pyometra. All strains (14/14) contained at least 1 antimicrobial resistance gene and 50% (7/14) of strains contained multiple resistance genes. There was no relationship between ability of biofilm formation and presence of multiple drug resistance genes. In total, 18 virulence factors were identified. Multiple virulence factors were present in all strains (14/14), including factors associated with uropathogenic *E. coli* in humans. There were no differences between the number or type of virulence factors present and the organism's ability to form biofilm. In conclusion, biofilm producing *E. coli* were more likely to be resistant to more antimicrobials than nonbiofilm producing bacteria. However, there were no relationships between the organism's ability to produce biofilm and presence of individual virulence factors or antimicrobial resistance genes. Surprisingly, all strains of *E. coli* evaluated were resistant to antibiotics commonly used in veterinary practice. This underlies the importance of antimicrobial stewardship and judicious antimicrobial use.

Keywords: *Escherichia coli*, biofilm, canine, pyometra, antimicrobial resistance