Detection of genes encoding multidrug resistance and biofilm virulence factor of uterine pathogenic bacteria in postpartum dairy cows

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Reckless use of antibiotics and/or development of biofilm are the rationale for the development of multidrug resistance (MDR) of pathogenic bacteria. The objective was to detect MDR genes in *Trueperella pyogenes* (16SrRNA, *intI*1, *intI*2, and cassette genes) and to detect biofilm virulence factor (VF) genes (*Agn43a* and *Agn43b*) in *Escherichia coli* isolated from the uterus of postpartum dairy cows. Uterine secretions from all parity postpartum Holstein cows (N=40) were collected using cytobrush technique following a sterile procedure as part of diagnosing cows with uterine inflammatory conditions. The cytobrush brush was stored in specimen collector (Diagnostic Systems, Sparks, MD), placed in a cooler with ice and transported to the laboratory within 2 h. The pathogens were isolated strictly following methods described. Initial identification was based on colony morphology and biochemical characteristics. Pure cultures were isolated. To further identify and classify the single species, and to determine the presence of MDR and VF genes, the genes fragments were amplified using the respective primers (NCBI) by either singleplex or multiplex polymerase chain reaction (PCR) protocol. Further, PCR products were run on agarose gel and viewed after ethidium bromide staining to make certain a single amplicon for a set of primer (Figure).

Figure. Photograph of the ethidium bromide-stained electrophoresis gel, with amplicons of the expected sizes

<table>
<thead>
<tr>
<th>16 s rRNA gene</th>
<th><em>IntI</em>1 gene</th>
<th>Gene cassette</th>
<th>Agn43aCFT073</th>
<th>Agn43bCFT073</th>
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*T. pyogenes* isolates were positive for the presence of *intI*1 gene and gene cassettes. Five cows were *intI*1 positive. Of those five, four cows were gene cassette positive. No cows were *intI*2 positive. The 1048 and 1608-bp amplicon revealed presence of *aadA*5 and *aadA 24-ORF1* gene, respectively. The *aadA*5 indicated resistance to sulfadiazine, bacitracin, florfenicol and ceftiofur. The *aadA 24-ORF1* indicated resistance to sulfadiazine, bacitracin, penicillin, clindamycin and erythromycin. The VF genes, *Agn43a* and *b* were present in *E. coli* isolates from persistently infected cows.

In conclusion, detection of *intI*1 and gene cassettes can be associated with integron-cassette mediated multidrug resistance in *T. pyogenes* isolates. Presence of VF genes indicated the formation of biofilm in the uterus of persistently infected cows possibly contributing to multidrug resistance in *E. coli* isolates.

**Keywords:** Dairy cows, postpartum uterus, bacteria, multidrug resistance, virulence factor