

A DIAGNOSTIC TOOL FOR THE DETECTION AND QUANTIFICATION OF PATHOGENIC BACTERIA IN BOVINE MILK SAMPLES FROM DAIRY FARMS IN BANGLADESH.

Pochodyla¹, M.; Solaiman², M.

¹HIPRA, Amer (Girona), Spain

²NASCO AGRO Products, Chittagong, Bangladesh

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INTRODUCTIONS

STARTCHECK[®] is a reliable diagnostic tool that uses a new methodology to collect milk samples and detect the major mastitis causative agents. STARTCHECK[®] can be used to complement Somatic Cell Count (SCC) and bacterial culture to monitor mastitis at a herd level¹. STARTCHECK[®] has been used worldwide since 2009 and milk samples from 44 different countries have been analysed. The aims of the present study were: 1) to show the results obtained from milk samples from Bangladesh dairy farms, using an FTA card and PCR diagnostic tool (STARTCHECK[®]) provided by HIPRA and 2) to show that STARTCHECK[®] can be used to complement Somatic Cell Count (SCC) and Bacterial culture to monitor mastitis at a herd level by detecting the major mastitis causative agents [*Staphylococcus aureus*, *Escherichia coli*, *Staph. Sp. (Coagulase Negative Staphylococci)* and coliform bacteria].

METHOD AND MATERIAL

A total of 102 Bulk Tank Milk (BTM) samples and Pooled Mastitis Milk (PMM) samples were collected from different dairy farms in Bangladesh, between July and August 2015. The samples were taken by professionals according to the STARTCHECK[®] instructions [Drawing up 250ul of milk in a sterile manner (gloved hands) from the Bulk Tank (after 5 minutes of stirring) and 250ul of milk from the pooled mastitis cows in separate pipettes, then impregnating the designated areas (BTM and Mastitis Pooled) on the FTA card with the respective milk samples]. The inoculated FTA cards were sent with the farm details (number of lactating cows, average milk production and most recent Bulk Tank somatic cell count [SCC] reading) to DIAGNOS (HIPRA) in Amer, Girona, Spain via courier services. (Fig. 1).



Figure 1. STARTCHECK[®] kit (Instructions, FTA card, pipette, plastic bag with desiccant and envelope).

The samples were processed and tested using the Real-Time Multiplex PCR assay, to detect the presence of genetic material for *S aureus*, *E. coli*, *Staph. Sp. (Coagulase Negative Staphylococci)* and coliform bacteria. The results were determined as positive or negative based on the Cycle threshold (Ct) values, with Ct values below 37 being considered as positive. The positive results were reported in a semi-quantitative manner, indicating the amount of genetic material obtained from each bacteria in the samples.

RESULTS

All samples (100%) were positive for at least one bacteria. *Staphylococcus aureus*, *Escherichia coli*, *Staph. Sp. (Coagulase Negative Staphylococci)* and coliform bacteria were detected in 33%, 93%, 99% and 96% of samples respectively. Of these 102 samples 51 were BTM samples and 51 were PMM samples. The prevalence of each pathogen in its respective

sample type is as follows:

Staph aureus (27% and 39%), *E. coli* (88% and 89%), *Staph. Sp. (Coagulase Negative Staphylococci)* (98% and 100%) and coliform bacteria (94% and 98%). Results for each specific pathogen are showed in figures 2- 5.

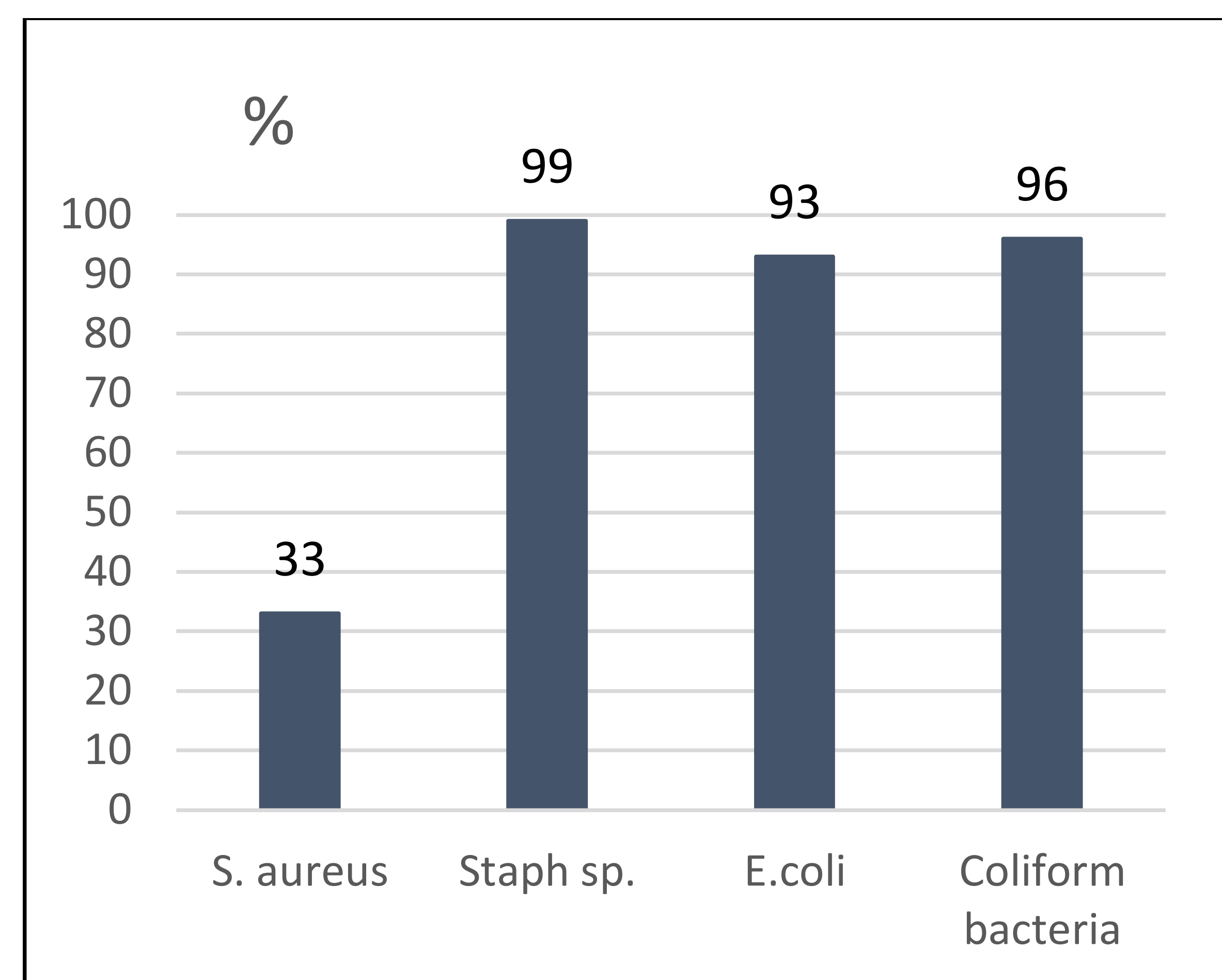


Figure 2. Percentage(%)of samples with positive PCR results to *Staphylococcus aureus*, *Escherichia coli*, *Staph. Sp. (Coagulase Negative Staphylococci)* and coliform bacteria.

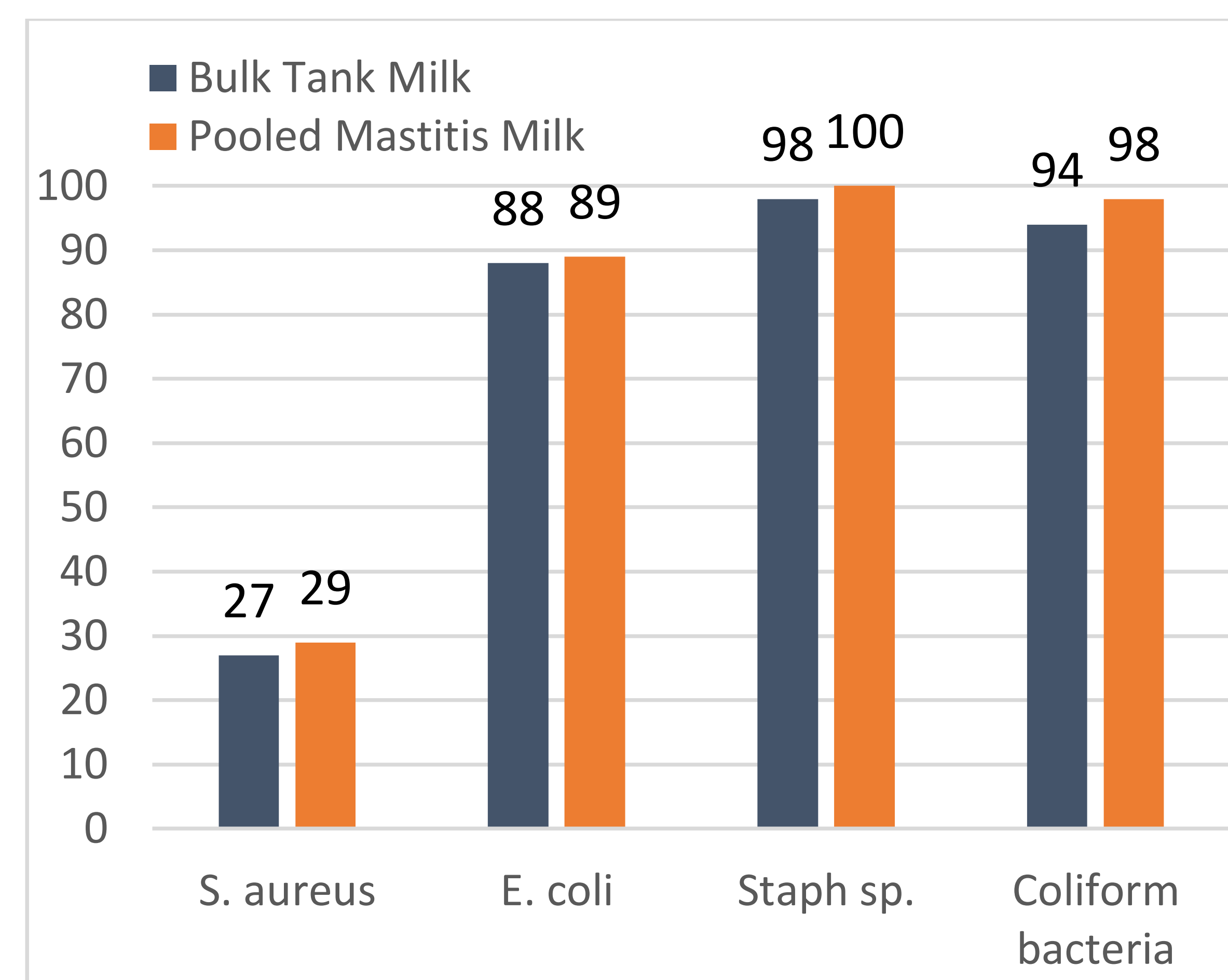


Figure 3. The prevalence (%) of *Staphylococcus aureus*, *Escherichia coli*, *Staph. Sp. (Coagulase Negative Staphylococci)* and coliform bacteria in its respective sample type.

DISCUSSION

STARTCHECK[®] is a good diagnostic and surveillance tool to identify pathogenic bacteria present at a herd level using BTM and PMM samples. The high specificity and sensitivity circumvents limitations experienced with bacteriology (transport or poor growth) and the human error with individual cow SCC cut off level of 200 000 cells/ ml.

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