The Prevalence of Environmental Pathogens Causing Mastitis in Dairy Cattle Reared Under Different Biosecurity Levels

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1. Abstract

The prevalence of contagious and environmental mastitogenic pathogens was investigated in dairy cattle farms which was categorized as poor, intermediate and high biosecurity levels. Those biosecurity levels were determined according to certain managemental practical procedures noticed in these farms. A total of 190 quarter milk samples were collected from 28 cows and 20 buffaloes from 6 farms in three governments in Egypt (El-Fayoum, Gharbia and Giza). Also, a total of 96 teat swabs were collected during this study, besides 40 swabs from milking cups (milking machine), 24 swabs from floor, 24 from wall and 18 from workers' hands. All milk and environmental samples were tested for total viable colony count and for presence of *Enterobacteriaceae*, *Staphylococcus* spp. and *Streptococcus* spp. Results showed that the mean of total colony count (TCC) was the lowest level in milk samples in intermediate than high and poor biosecurity farms. All environmental samples showed the lowest mean of TCC in high biosecurity farms, while poor and intermediate were nearly the same. Coagulase–positive staphylococcus was isolated from different samples collected from the dairy farms. Teat samples in high biosecurity farms showed the highest risk for coagulase–negative staphylococcus followed by milking cups in poor biosecurity farms. Teat, wall and floor samples in high biosecurity farms were highly contaminated with *E. coli*. *Citrobacter*, *Klebsiella* and *Proteus* were isolated from different samples. milking cups showed the highest load for *Enterococcus* in high biosecurity farms. Milking cup in high biosecurity farms presented the highest risk for *Streptococcus dysgalactiae*, while in intermediate biosecurity farms it was highest in milk samples. Odds ratio is indicated for risk assessment, while Cramer's v and partial omega square are indicated for practical importance. It was concluded that biosecurity programs should be adopted by dairy producers to prevent the chance of mastitis. The biosecurity program needs continuous evaluation at all levels to discover any fault in its application.

Key words: Environmental mastitis; TCC; Coagulase – positive staphylococcus; *Enterococcus*; Cramer's v; Partial omega square.

2. Introduction

One of the most serious illnesses affecting dairy cows worldwide is still bovine mastitis [1, 2]. Mastitis causes significant direct and indirect financial losses. The total losses clearly encompass direct expenses related to the disease's treatment. However, the indirect expenses such as the need to discard mastitic milk, the loss of future milk output and quality, and the heightened

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risk of culling are even more substantial and cannot be ignored. Also, several bacteria causing mastitis can infect humans, particularly those with weakened immune system [3].

Mastitis is an inflammation of the mammary glands that results in changes to the glandular secretions and gland tissue, which can reflect physically and chemically, respectively. Bacterial infections that penetrate the udder and trigger immunological reactions are frequently cause for concern. The disease can present in both clinical and subclinical forms, the later are more troubling since it is silent, resulting in infections that go unnoticed and ongoing problems with the quality of milk [4]. bacterial pathogens are divided into several groups which are environmental and contagious bacteria [5].

Contagious pathogen, as the primary routes of transmission between infected and uninfected udder quarters, they are typically discovered on the udder or teat surface of infected cows, generally during milking [6]. This group of organisms include *Corynebacterium bovis* and *Mycoplasma bovis* which are less common causes of infection, while *Streptococcus agalactiae*, and *Staphylococcus aureus* (Coagulase Positive Staphylococci, CPS) are the most common causes [6]. Environmental pathogen come from the cow's surroundings (bedding, manure, water, and soil) and infect the udder when the teat comes in contact with contaminated materials. Gram-positive organisms include environmental pathogens such as *Enterococcus faecalis*, *Enterococcus uberis*, and *Enterococcus equinus*. *Enterobacter* species, *Serratia species*, *Pseudomonas* species. *Klebsiella* species, and *Escherichia coli* are examples of gram-negative organisms [7].

There is a strong link between contagious and environmental mastitis pathogens, as some bacteria can act in both ways under certain conditions Some environmental pathogens (*S. uberis, S. dysgalactiae*) can behave like contagious pathogens, spreading from cow to cow in unhygienic milking conditions [8, 9]. *Staph aureus* that contaminates the milk is mostly isolated from the udder and teat apices [10, 11]. Coliforms are accompanied with poor udder hygiene, uncleaned stalls, bedding materials and milking machine [12]. Poor environmental conditions can increase the risk of contagious mastitis, as stressed cows are more susceptible to infection [13]. Both types require proper hygiene, cow management, and biosecurity measures to reduce mastitis incidence [14, 15].

Being a multifactorial disease, mastitis is typically caused by the interaction of number of variables related to the host, pathogen(s), environment, and management [12, 16, 17]. Several risk factors contribute to mastitis occurrence, including farm management practices, milking procedures, cow immunity, and environmental conditions [18]. Bedding, housing, and milking techniques, pre and post teat dip, cleaning and disinfection of milking parlor are examples of environmental factors [16]. The contaminated surroundings or the infected udder are the source of the infection. The main sources of diseases and ways they spread are dirty udders and quarters, tainted teat cups, milking machines, worker's hands, and laundry [12]. The prevalence of pathogens that cause mastitis differs by location and management techniques [19].

A collection of management techniques or precautions to stop the introduction and spread of infections both within and between farms is known as biosecurity [20, 21]. Biosecurity has been

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described as the least expensive method of managing illnesses in herds or flocks [22]. When fighting endemic illnesses in a country or preparing for emerging diseases, knowledge about the farms' degree of biosecurity is crucial. It also helps determine if and where development of biosecurity is required [23]. Farm evaluations can be done by qualified assessors who monitor the physical infrastructure, biosecurity measures, and animal health care procedures in the dairy farm [24, 25]. Quantifiable indicators, such as pathogen testing or disease prevalence, are used to determine how far the biosecurity procedures affect the health of animals [26, 27]. The microbiological evaluation of milk is an important factor in determining its safety and quality and is a reflection of farm cleanliness [28]. Biosecurity in dairy farms emphasis on five categories: animal, feed, waste, sanitary, and structural [29]. A successful biosecurity program must be more than just a list of tasks. It must be adaptable to the particular circumstances of each farm, necessitating knowledge of disease control objectives, biosecurity principles, and particulars of the biology and epidemiology of specific infections. Designing efficient control and preventive methods requires an understanding of the prevalence of infections that cause mastitis and the risk factors that are linked to them [30].

Therefore, the aim of this work is to investigate the possible incriminated microorganisms that may cause mastitis in dairy cattle reared under different biosecurity levels. Such insights can guide farmers and veterinarians in implementing targeted interventions to minimize the impact of mastitis on dairy production.

3. Materials and Methods

3.1. Study Area and Population

A total of 190 quarter milk samples were collected from 28 cows and 20 buffaloes from 6 farms including smallholder, free stall) types. In three governments in Egypt (El-Fayoum, Gharbia and Giza).

3.2. Sample Collection and Laboratory Analysis

3.2.1. Milk samples

Sample collection and the California mastitis test (CMT) was performed on the farm according to Quinn et al [31].

Quarters milk samples (QMS) were collected from every cow after thorough physical examination. Examining the udder and teats for any inflammation, the animals' temperature, and the milk's quality for the presence of clots, blood, and flake were used to diagnose mastitis in the animals [32].

CMT was used to screen subclinical mastitis (SCM) after a physical examination of apparently healthy animals. According to National Mastitis Council (NMC) [33]. CMT findings were subjectively evaluated as negative, trace, 1+, 2+, or 3+. Cows with CMT readings of (1+, 2+, or 3+) were classified as positive for SCM, whereas negative and trace values were regarded as negative. Next, the cows were grouped, and either mastitic or non-mastitic findings were noted. If CMT proved positive in at least one quarter, the cow was considered mastitis positive. After that, samples were taken from cows who tested positive for CMT.

The udder was properly cleaned and dried before sampling where, five to ten ml of milk was aseptically collected from each quarter after the teats were cleaned with 70% ethyl alcohol swabs and 4–5 streams of milk were stripped.

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The milk was then placed in individual universal bottles that were positioned slightly horizontally to prevent contamination from the udder [32, 33].

3.2.2. Environmental samples

3.2.2.1. Teat swabs

A total of 96 teat swabs were collected during this study. A sterile cotton swab dampened in buffered peptone water was used to collect teat swabs. The procedure involved swabbing one side of the teat barrel from top to bottom, covering the teat end, and then swab the other side from top to bottom. Swabs were cooled right away after being submerged in 4 ml. of buffered peptone water [34, 35]. Four teat swabs collected from each animal, where 2 swabs were pooled for bacteriological isolation and the other 2 were pooled for TCC. Total bacterial count (TBC), gram-negative coliforms, Streptococcus species, and Staphylococcus species were examined in teat swabs as well [34].

3.2.2.2. Milking cup (milking machine) swabs

A total of 40 milking cup samples were collected within teat cups, sampling was taken from the top (about 1–1.5 cm deep) and from the bottom (around 10–12 cm deep). The whole circle of the inner wall of the teat cup was swabbed in a circular pattern. Four sterile swabs were used in each milking cup unit, where 2 swabs were pooled for TCC and the other 2 were pooled for isolation. The swabs were then submerged in a transportation medium and subjected to a bacteriological analysis [36].

(N.B: during swabbing from milking machines, accumulation of moisture as a remnant of rinsing water was noticed.)

3.2.2.3. Swabs from wall and floor

A total of 48 samples where, 8 swabs were collected from each farm 4 samples from walls and 4 samples from floors. The whole 100 cm^2 surface area was rotated axially and laterally in a zigzag pattern. The swabs were then submerged in transportation medium (physiological saline for TCC and BHI broth for isolation) [37].

3.2.2.4. Swabs from workers' hands

A total of 18 hand swabs were collected from the investigated dairy farms where, the swabs were collected from 3 persons in each farm. Each hand was swabbed using a cotton swab soaked in sterile solvent containing normal saline, with each hand's five fingers extended. The dorsal side of the finger, the palm, the inter digital region, the finger's palm side, and the hand's dorsum were among the sample locations. Two swabs from each hand were collected, then one swab was submerged in saline for TCC and the other one was submerged on BHI broth for isolation [38, 39].

3.2.3. Bacteriological isolation and identification

Following the guidelines provided in the Laboratory Handbook on Bovine Mastitis [33], all milk and environmental samples were tested for total viable colony count by spread plate method [40]. Also, all milk sample and environmental swabs were tested for presence of *Enterobacteriaceae*, *Staphylococcus* spp. and *Streptococcus* spp.

Aseptically, only 0.01 ml aliquot of every milk sample was streaked onto the 5% sheep blood agar, MacConkey agar, mannitol salt agar and Edward medium modified with 7% sheep blood

agar plates. Plates were incubated aerobically for 18 to 24 hours at 37°C.

Growth characteristics, the catalase test, tube coagulase tests, and mannitol salt agar were used to identify the species of *Staphylococcus*. The catalase test and growth characteristics on Edward's media and KF media were used to identify the species of *Streptococcus*, *Enterococcus* and the CAMP test was used to differentiate the bacteria within the group. Colony morphology was used to identify gram negative bacteria.

MacConkey lactose fermentation, oxidase test, IMVC tests and TSI (triple sugar iron agar), colony morphology on MacConkey agar and EMB media were used for identification of *Enterobacteriaceae*.

3.3. Data Collection on Risk Factors and Biosecurity Level

Data were collected during the farm visits and in-person interviews to determine risk factors and biosecurity levels at each farm. The data gathered comprised farm management practices were detailed in table (1).

3.4. Data Analysis

Data analysis was performed using SPSS® ver. 27 (SPSS, Inc., Chicago. Il. USA).

a) Statistical Significance in means and proportions between groups were estimated using ANOVA and Chi-square test, respectively. significance level of $\alpha \leq 0.05$ was used.

b) Epidemiological parameters of risk and effect size were measured by Cramer's v, partial omega-square and odds ratio.

c) The interpretation of the odds ratio (OR) value as follows: [41, 42].

- If odds =1: the event is equally likely to occur or no chance (no association between exposure and outcome).

- Odds >1: the event more likely occur than not (positive association between exposure and outcome.

- Odds < 1: the event is less likely occur than not (negative association or a protective effect of the exposure on the outcome).

d- Cramer's v interpretation was as follows: [43, 44].

- From 0 to 0.06 = small effect

- From 0.07 to 0.17 = intermediate effect, from 0.18 to 0.29 = large effect.

e- Partial omega-square interpretation was as follow: [43, 45].

- From 0.01 to 0.05 = small effect,

- From 0.06 to 0.13 = medium, from 0.14 to more = large effect.

Statistical significance refers to whether a result is due to chance or variability in the samples whereas practical significance (effect size) refers to whether the result is useful in real world [43].

4. Results

In table (2) after, milk samples with the lowest mean were noticed in intermediate biosecurity farms (2.58 ±0.43) and the lowest mean noticed in high biosecurity farms in teat samples (2.96 ±0.42).

Result of all environmental samples taken from farms which had the highest biosecurity were found to have the lowest mean TCC compared to those with poor or intermediate biosecurity levels (P<.05) (table, 3). Except for

milking cups the lowest mean was noticed in high biosecurity farms (3.46^{a} ±0.52) followed by poor farms (4.26^{b} ±0.27) and the highest mean was noticed in intermediate farms (4.74^{c} ±0.33). Practical importance of all samples according to partial omega square is large.

Regarding coagulase-positive staphylococcus it was isolated with highest level from hand samples in poor biosecurity farms (91.7%), followed by milking cup samples in high biosecurity farms (80%), while the lowest level (25%) appeared in teat samples (intermediate biosecurity) and in hand samples (high biosecurity) farms. Also, there was a significant statistical difference (*P*= 0.002) noticed in milk and hand samples of poor biosecurity farms (the highest) in comparison with intermediate and high farms. Additionally, a significant statistical difference appeared in teat samples of high biosecurity farms (the highest) in comparison with intermediate and poor farms (*P*= 0.003) (Figure 1).

Following coagulase-negative staphylococcus it was isolated with highest level from teat samples in high biosecurity farms (70%) followed by milking cup samples (50%) in poor biosecurity farms, while the lowest level (0%) in (teat, milking cup, hand, wall and floor) in intermediate biosecurity farms and hand, floor samples in high biosecurity level farms. Also, there was a significant statistical difference noticed in teat samples of high biosecurity farms (the highest) in comparison with other biosecurity level farms (*P*= 0.000). Also, there was a significant statistical difference noticed in milk samples of poor biosecurity farms (the lowest) in comparison with other biosecurity farms (*P*= 0.001) (Figure 2).

E. coli was isolated with highest level from teat samples in high biosecurity farms (50%), followed by wall and floor samples in high biosecurity farms (42.9%), while the lowest level showed in wall, floor (0%) in poor biosecurity farms. Also, there was a significant statistical difference noticed in milk samples of intermediate biosecurity farms (the highest) in comparison with poor and high biosecurity farms (*P*= 0.000), additionally there was a significant statistical difference appeared in teat, wall and floor samples (the highest) of high biosecurity farms (*P*= 0.008) (Figure 3).

Also, *Citrobacter* was isolated with highest level from wall and floor (100%) in intermediate biosecurity farms, followed by milking cups (83.3%) in poor biosecurity farms, while the lowest in milk (0%) in high biosecurity farm. Also, there was a significant statistical difference in milk and milking cup in poor biosecurity farms (the highest) in comparison with other biosecurity levels farms at (*P*= 0.000). Additionally, there was a significant statistical difference appeared in teat samples of intermediate biosecurity farms (the lowest) in comparison with other biosecurity levels farms (*P*= 0.02) (Figure 4).

In the same way, *Klebsiella* was isolated with highest level from hand samples (83.3%) of poor biosecurity farms, followed by wall and floor samples (75%) of intermediate biosecurity farms, while the lowest in

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milk and milking cup (0%) of intermediate biosecurity farms and hand samples (0%) of high biosecurity farms. Also, there was a significant statistical difference in milk, hand (the highest) and floor samples (the lowest in poor biosecurity farms) in comparison with intermediate and high biosecurity farms (*P*= 0.003). Additionally, there was a significant statistical difference appeared in teat samples of intermediate biosecurity farms (the highest) in comparison with other biosecurity levels farms (*P*= 0.008) (Figure 5).

On the other hand, *Proteus* was isolated with highest level from milking cup in poor biosecurity farms (41.7%) followed by milking cups in high biosecurity farms (40%), while the lowest in milk of high biosecurity farms and milking cup, wall and floor of intermediate biosecurity farms. Also, there was a significant statistical difference in milk samples of high biosecurity farms (the lowest) in comparison with other biosecurity level farms (*P*= 0.002) (Figure 6).

Moreover, *Enterococcus* was isolated with highest level from milking cups of high biosecurity farms (80%), followed by wall and floor of intermediate biosecurity farms (75%), while the lowest was in milk of intermediate biosecurity (13%). Also, there was a significant statistical difference in milk samples of poor biosecurity farms (the highest) in comparison with intermediate and high biosecurity level farms (*P*= 0.000) (Figure 7).

Streptococcus dysgalactiae isolated with highest level from milk samples of intermediate biosecurity farms (%52.2), followed by milking cup in high biosecurity farms (40%), while the lowest in hand, wall and floor of high biosecurity farms. Also, there was a significant statistical difference in milking cup of high biosecurity level (the highest) in comparison with intermediate and poor biosecurity farms. Additionally, there was a significant statistical difference noticed in milk of intermediate biosecurity farms (the highest) in comparison with other biosecurity levels farms (*P*= 0.000) (Figure 8).

Summarizing the effect size of biosecurity levels on the isolated microorganisms. For odds in milk samples, the opportunity of presence of *Streptococcus dysgalactiae* is 10 times more likely in intermediate than poor and high biosecurity farms, followed by *E. coil* which is 9.4 times in intermediate biosecurity farms as shown in table (4).

In teat samples, the probability of isolation of *E. coli* is 48 times more likely in high than poor biosecurity farms and 9.6 times in intermediate than poor biosecurity farms. Coagulase- negative staphylococcus is 12 times more likely in high than poor biosecurity farms. *Klebsiella* is 11.8 times more likely in intermediate than poor and high biosecurity farms. Coagulase- positive staphylococcus is 4.4 times more likely in high than poor and intermediate biosecurity farms. *Proteus* and *Enterococcus* are 2.6 times more likely in high than poor and intermediate biosecurity farms. While *Streptococcus dysgalactiae* is 3 times more likely in intermediate than poor and intermediate biosecurity farms.

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In milking cups, the possibility of the presence of coagulase- positive staphylococcus is 8 times more likely in high than poor biosecurity farms and is 2 times in intermediate biosecurity farms. *E. coli* is 5.5 times more likely in intermediate than poor biosecurity farms and 2.8 times more likely in high than poor biosecurity farms.

In walls, the chance of isolation of *Streptococcus dysgalactiae* is 6.33 times more likely in intermediate than poor and high biosecurity farms. Followed by *Enterococcus* which is 5.6 times in intermediate than poor biosecurity farms and 1.4 times more likely in high than poor biosecurity farms, while *Proteus* is 3.2 times more likely in high than poor biosecurity farms.

In floor, the hazard of the presence of *Klebsiella* is 19.5 time more likely in intermediate than poor biosecurity farms and 8.7 time more likely in high than poor biosecurity farms. Coagulase positive *staphylococci* is 4.5 time more likely in intermediate than poor biosecurity farms and 2 times more likely in high than poor biosecurity farms. *Proteus* is 2.3 times more likely in high than poor and intermediate biosecurity farms. *Enterococcus* is 2 time more likely in intermediate than poor and high biosecurity farms.

All odds ratio above 1 was considered risk factor and below 1 was considered protective effect.

Practical importance of all result in form of Cramer's v was large except for milk sample and coagulase - positive staphylococcus the practical importance found to be medium.

Table (5) showed that there was a statistically significant difference between categories of infection and biosecurity levels in milk samples. Single and two mixed infections appeared more in high than poor and intermediate biosecurity farms. In poor biosecurity farms, the occurrence of more than 3 mixed infection is the highest compared to other biosecurity level's farms.

For odds in milk samples, the opportunity of isolation of three mixed infection was 1.5 times more likely in intermediate than poor and high biosecurity farms.

In teat sample, there was a statistically significant difference between categories of infection and biosecurity levels with more than 3 infections appeared more in high than intermediate and poor biosecurity farms.

For odds in teat samples, the opportunity of isolation of two and three mixed infection was 1.6 times more likely in intermediate than poor and high biosecurity farms. In high biosecurity farms, the opportunity of isolation of more than three mixed infection was 3.6 time more likely than poor and intermediate biosecurity farms.

Practical importance in form of Cramer's v in milk and teat samples was large.

Table (6) indicated that there was no statistically significant difference between any of biosecurity level and categories of infections in all environmental samples except for wall where, there was a statistically significant difference between poor and high biosecurity farms. The two mixed infection occurred more in high than poor biosecurity farms.

For odds any value below 1 consider protective value not risk, so that in milking cups the opportunity of occurrence of three mixed infection was1.4 times more likely in intermediate than poor and high biosecurity farms.

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Two mixed infection was 2.4 times more likely in high than intermediate and poor biosecurity farms. More than three mixed infection was 1.7 times more likely in high than intermediate and poor biosecurity farms.

In hand, the opportunity of occurrence of two and three mixed infection was 1.7 times more likely more in high than poor and intermediate biosecurity farms.

In wall, the risk of occurrence of more than three mixed infection was 1.8 times more likely in intermediate biosecurity farms in comparisons with poor and high farms.

In floor, the risk of occurrence of three mixed infection was 1.8 times more likely in high than poor and intermediate biosecurity farms.

Practical importance in all environmental samples in form of Cramer's v found to be large.

5. Discussion

This work was done in dairy cattle farms reared under different hygienic measures to demonstrate the effect of biosecurity levels on the prevalence of opportunistic pathogens which may be a threaten to cause mastitis at any time.

Regarding our TCC results, it was declared that the mean of TCC was the lowest in milk samples in intermediate than high and poor biosecurity farms. These findings may be attributed to that some farms have high biosecurity level but may have a defect in application of any step related to biosecurity program such as application of disinfection, so that any biosecurity program needs continuous evaluation and monitoring to discover any fault in its application. The mean of TCC in teat samples showed the lowest level in high than poor and intermediate biosecurity farms (Table 2).

In table (3), all environmental samples showed the lowest mean of TCC in high biosecurity farms, while poor and intermediate were nearly the same, except in milking cup samples the highest mean of TCC was recorded in intermediate biosecurity farms and this may be due to fault in sanitation and disinfection of milking cups.

Partial omega square in both milk and environmental samples was large and this indicate the practical importance for application of biosecurity program to decrease the burden of TCC. These findings agree with Laban et al. [46] who found that cleaning and sanitation methods impacted the microbial reduction obtained differently. Also agree with Myllys and Rautala, [47] who found that poor milking hygiene has been associated with inferior milk quality.

In figure (1), the isolation of coagulase – positive staphylococcus from different samples collected from the dairy farms indicated an imperfection in the biosecurity program even it was classified as high, intermediate, or poor level. Hand samples in poor biosecurity farms showed the highest threaten for this microorganism and, for milking cup samples in high biosecurity farms, this finding make a focus on some points or details in biosecurity program, which mean the need to adjust or addition of some biosecurity points as hand washing and disinfection of milking cups. This result was in agreement with Silva et al. [48].

Results from figure (2) indicated that teat samples in high biosecurity farms showed the highest risk for coagulase – negative staphylococcus followed by milking cups in poor biosecurity farms. This may be due to

misuse of teat dip, or the teat dip was not suitable for this microorganism. For milking cups this may be as a result of improper disinfection of milking cups. It was noticed that this microorganism isolated only from milk samples in intermediate biosecurity farms. This finding agrees with Lim et al. [49] and Thorberg et al. [50].

In figure (3), teat wall and floor samples in high biosecurity farms, were highly contaminated with *E. coli* and this may be owing to improper disinfection of milking parlor and sanitation of teat tips. Also, milk samples in intermediate biosecurity farms showed heavily contamination by this microorganism.

In figure (4), milk and milking cup samples in poor biosecurity farms showed the highest threaten for *Citrobacter*, while in intermediate biosecurity wall and floor warn for this microorganism. This may be because of poor biosecurity program and management.

In figure (5), hands in poor biosecurity farms indicate the highest risk for *Klebsiella*, while teats, wall and floor in intermediate biosecurity farms warn for this microorganism. This may be because of poor biosecurity program and management.

In figure (6), milking cups showed the highest warn for *Proteus* in poor and high biosecurity farms and this may be due to moisture that accumulated in milking cups and lack of disinfection or sanitation.

Data of figures (3, 4, 5 and 6) showed agreement with El-Mokadem et al. [51] and Saidani et al. [52].

In figure (7), milking cups showed the highest load for *Enterococcus* in high biosecurity farms, while this microorganism was mostly isolated from wall and floor in intermediate biosecurity farms and milk sample in poor biosecurity farms. This may be attributed to an imperfection in biosecurity program at all levels. This conclusion agree with Juliano et al. [53].

In figure (8), milking cup in high biosecurity farms presented the highest risk for *Streptococcus dysgalactiae,* while in intermediate biosecurity farms it was highest in milk samples. This may be because of misapplication of disinfection program. This data agree with Wente and Krömker [54].

Table (4) indicated the epidemiological importance of isolated microorganisms from milk and environmental samples collected from dairy cattle farms with different biosecurity levels, where odds ratio is indicated for risk assessment, while Cramer's v is indicated for practical importance. This result throws a light on the importance of effective cleaning and sanitation programs to decrease the load of contamination.

Table (5) showed categories of infection in different biosecurity levels in milk samples. Single and two mixed infections were recorded in high biosecurity farm, while more than 3 mixed infection was recorded in poor biosecurity farms. This result guides us to the need of use broad spectrum and specific disinfectant in poor biosecurity farms to combat the challenge of mixed infection. In teat samples more than 3 mixed infection was recorded in high biosecurity farms and this may be attributed to fault in disinfecting procedure of milking cups and teat. Based on result of Cramer's v, it's important to make effective biosecurity program with regular evaluation to reduce the load of infection.

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Results in table (6) showed that wall samples in high biosecurity farms hold two mixed infections more than poor biosecurity farms. This may be resulted from improper disinfection of milking parlor. Although there was no statistically significant difference between environmental samples and categories of infection, result of Cramer's v was large. This finding prove that cleaning and disinfection of milking parlor environment is critical for decreasing the burden of infections. These finding including tables (4, 5 and 6) showed agreement with Hutchison et al. [55] who found that reducing the environmental pathogen contamination of the teat end is a method for controlling environmental mastitis. Also, machine milking is a significant cause of bacterial cross-contamination from cow to cow.

Veterinarians have generally advised pre- and post-milking teat disinfection, and dairy producers are becoming more following this advice. This process is easy to use, cost-effective, and efficient in reducing the spread of infectious mastitis pathogens. More recently, the rate of intramammary infections by environmental pathogens was decreased by teat dipping in conjunction with proper udder preparation.

Conflict of interest: Nothing to declare

7. References

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Table (1): The biosecurity level among dairy cattle farms in this study

Management practice / Milking technique	High	Intermediate	Poor		
	Automatic	Automatic	Manual	Automatic	Manual
Milking technique	daily with water and detergent	daily with water only	daily with water only	daily with water only	daily with water only
Bedding (yes/no)	Yes	Yes	Yes	Yes	No
Drying towel (yes/no)	Yes	Yes	No	No	No
Towel for each cow (yes/no)	Yes	Yes	No	No	No
Washing hand between milking (yes/no)	Yes	No	No	No	No
Wearing gloves during milking (yes/no)	No	No	No	No	No
Teat dip (yes/no)	Yes	Yes	No	No	No
Testing of mastitis (yes/no)	Yes	No	No	No	No
Disinfection of milking parlor (yes/no)	Yes	Yes	No	No	No
Culling (yes/no)	Yes	No	No	No	No
Vaccination (yes/no)	Yes	No	No	No	No

Table (2): Total colony count (expressed as CFU/ml*10^2) in milk and teat samples collected from dairy cattle farms with different biosecurity levels

Sample	Biosecurity level	N	Mean ± SD.	Omega2
Milk	Poor	28	4.13^{c} ± 0.33	0.646
	Intermediate	6	2.58^{a} ±0.43	
	High	14	3.38^{b} ±0.49	
Teat	Poor	26	4.22^{b} ±0.39	0.684
	Intermediate	6	4.53^{b} ±0.47	
	High	14	2.96^{a} ±0.42	

Different superscript indicate significance at $p<0.05$

Table (3): TCC (expressed as CFU/ml*10^2) in (milking cups, hand, wall and floor samples) collected from dairy cattle farms with different biosecurity levels

Sample	Biosecurity level	N	Mean ± SD.	Omega2
Milking cups	Poor	12	4.26^b ±0.27	0.578
	Intermediate	3	4.74^c ±0.33	
	High	5	3.46^a ±0.52	
Hand	Poor	13	4.31^b ±0.39	0.777
	Intermediate	2	4.52^b ±0.32	
	High	3	2.26^a ±0.59	
Wall	Poor	13	4.19^b ±0.42	0.149
	Intermediate	4	4.22^b ±0.32	
	High	4	3.35^a ±1.23	
Floor	Poor	13	4.62^b ±0.33	0.439
	Intermediate	4	4.59^b ±0.26	
	High	6	3.84^a ±0.48	

Different superscript indicate significance at $p < 0.05$

Table (4): Epidemiological importance of the isolated microorganisms from milk and environmental samples collected from dairy cattle farms under different biosecurity levels

Samples		Milk		Teat		Milking cup		Hand		Wall		Floor	
Biosecurity levels		Intermediate	High	Intermediate	High	Intermediate	High	Intermediate	High	Intermediate	High	Intermediate	High
Coagulase - positive staphylococcus	odds	0.67	0.57	0.63	4.39	2	8	0.09	0.03	1	0.4	4.5	2
	Cramer's v	0.13		0.36		0.37		0.63		0.18		0.26	
Coagulase - negative staphylococcus	odds	1.41	0.22	Nil	11.96	Nil	0.25	Nil	Nil	Nil	0.67	Nil	Nil
	Cramer's v	0.25		0.58		0.47		0.30		0.18		0.52	
E. coli	odds	9.36	0.41	9.6	48	5.5	2.75	Nil	Nil	Nil	Nil	Nil	Nil
	Cramer's v	0.35		0.55		0.28		Nil		0.60		0.59	
Citrobacter	odds	0.07	Nil	Nil	0.75	0.04	0.05	0.71	0.24	Nil	0.27	Nil	0.08
	Cramer's v	0.43		0.24		0.65		0.30		0.42		0.58	
Klebsiella	odds	0.41	0.08	11.75	0.81	Nil	0.35	0.2	Nil	3	0.17	19.5	8.67
	Cramer's v	0.43		0.37		0.40		0.75		0.37		0.54	
Proteus	odds	1.94	Nil	Nil	2.61	Nil	0.93	Nil	Nil	Nil	3.17	Nil	2.33
	Cramer's v	0.23		0.15		0.39		Nil		0.19		0.17	
Enterococcus	odds	0.09	0.23	0.52	2.61	0.36	0.86	0.47	0.47	5.58	1.39	2	0.89
	Cramer's v	0.39		0.22		0.33		0.18		0.27		0.12	
Streptococcus dysgalactiae	odds	10.01	0.53	2.93	0.63	Nil	Nil	Nil	Nil	6.33	Nil	4.67	Nil
	Cramer's v	0.42		0.18		0.47		0.47		0.30		0.29	

Table (5): Categories of infection in different biosecurity levels in milk and teat samples

Sample	Biosecurity level		Infection				Total	Cramer's v
			Single	2 mixed	3 mixed	>3 mixed		
Milk	Poor	Count (%)	Nil	Nil	7 (25%)	21*(75.0%)	28	0.571
	Intermediate	Count (%)	Nil	Nil	2 (33.3%)	4 (66.7%)	6	
		odds	Nil	Nil	1.50	0.67		
	High	Count (%)	6*(42.9%)	4*(28.6%)	2 (14.3%)	2 (14.3%)	14	
		odds	Nil	Nil	0.50	0.05		
Teat	Poor	Count	2 (7.7%)	9 (34.6%)	10 (38.5%)	5 (19.2%)	26	0.293
	Intermediate	Count (%)	Nil	3 (50.0%)	3 (50.0%)	Nil	6	
		odds	Nil	1.67	1.60	Nil		
	High	Count (%)	Nil	2 (15.4%)	5 (38.5%)	6*(46.2%)	13	
		odds	Nil	0.30	1	3.60		

Asterisk indicate significant difference at $p<0.05$

Table (6): Categories of infection in different biosecurity levels in environmental samples

Sample	Biosecurity level		Infection				Total	Cramer's v
			Single	2 mixed	3 mixed	>3 mixed		
Milking cups	Poor	Count (%)	Nil	1 (8.3%)	7 (58.3%)	4 (33.3%)	12	0.189
	Intermediate	Count (%)	Nil	Nil	2 (66.7%)	1 (33.3%)	3	
		odds	Nil	0	1.43	1		
	High	Count (%)	Nil	2 (18.2%)	4 (36.4%)	5 (45.5%)	11	
		odds	Nil	2.44	0.34	1.67		
Hand	Poor	Count (%)	Nil	7 (53.8%)	3 (23.1%)	3 (23.1%)	13	0.425
	Intermediate	Count (%)	Nil	Nil	Nil	2 (100.0%)	2	
		odds	Nil	Nil	Nil	Nil		
	High	Count (%)	Nil	2 (66.7%)	1 (33.3%)	Nil	3	
		odds	Nil	1.71	1.67	Nil		
Wall	Poor	Count (%)	Nil	Nil	11(64.7%)	6 (35.3%)	17	0.419
	Intermediate	Count (%)	Nil	1 (25.0%)	1 (25.0%)	2 (50.0%)	4	
		odds	Nil	Nil	0.14	1.83		
	High	Count (%)	Nil	2*(40.0%)	3 (60.0%)	Nil	5	
		odds	Nil	Nil	0.82	Nil		
Floor	Poor	Count (%)	Nil	4 (26.7%)	8 (53.3%)	3 (20.0%)	15	0.436
	Intermediate	Count (%)	Nil	Nil	2 (50.0%)	2 (50.0%)	4	
		odds	Nil	Nil	1	1		
	High	Count (%)	1 (14.3%)	Nil	6 (85.7%)	Nil	7	
		odds	Nil	Nil	1.75	0.16		

Asterisk indicate significant difference at $p<0.05$

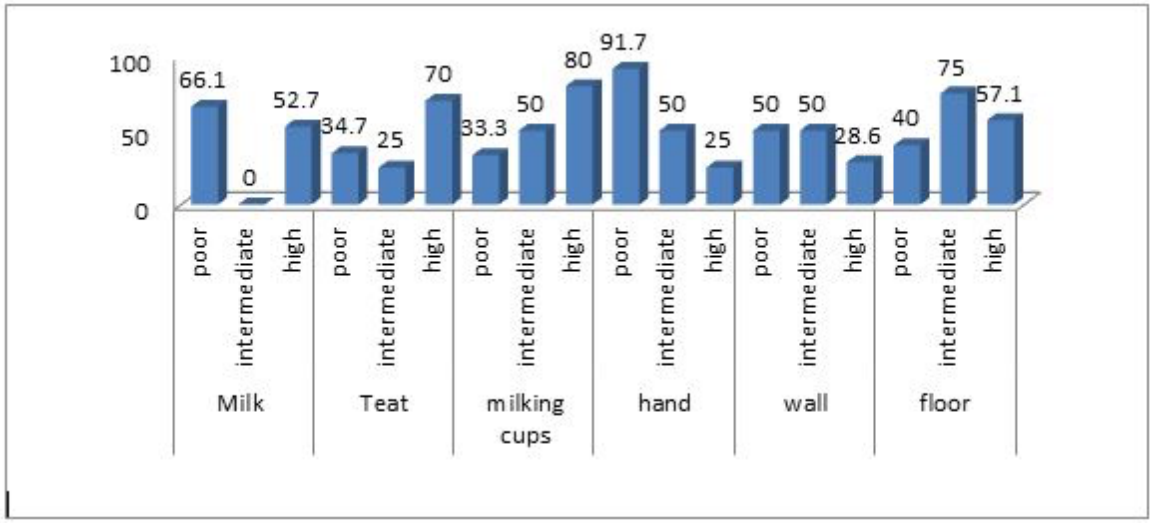

Fig. 1: Prevalence of coagulase - positive staphylococcus from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.

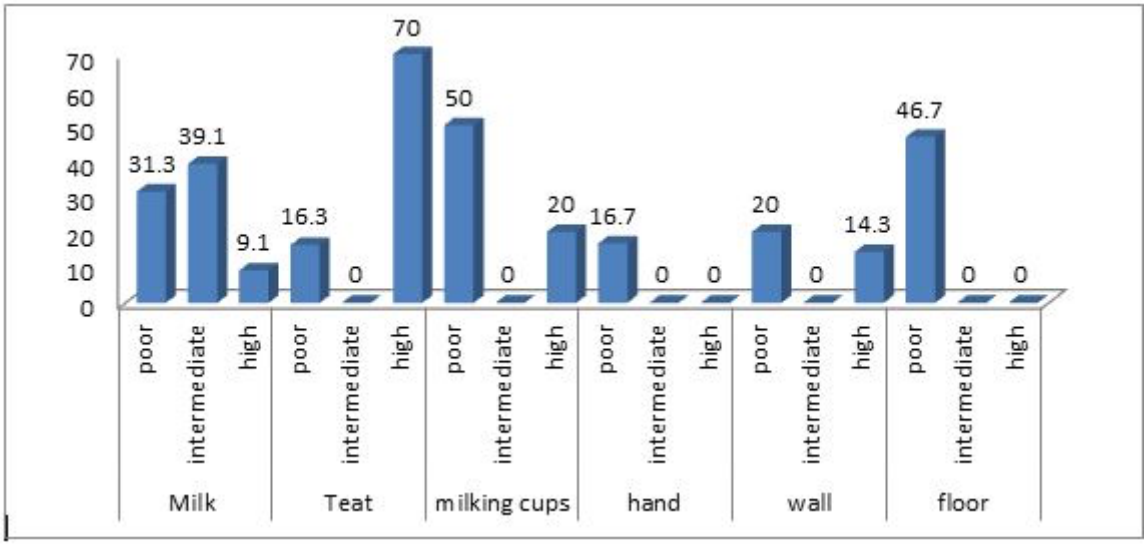

Fig. 2: Prevalence of coagulase - negative staphylococcus from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.

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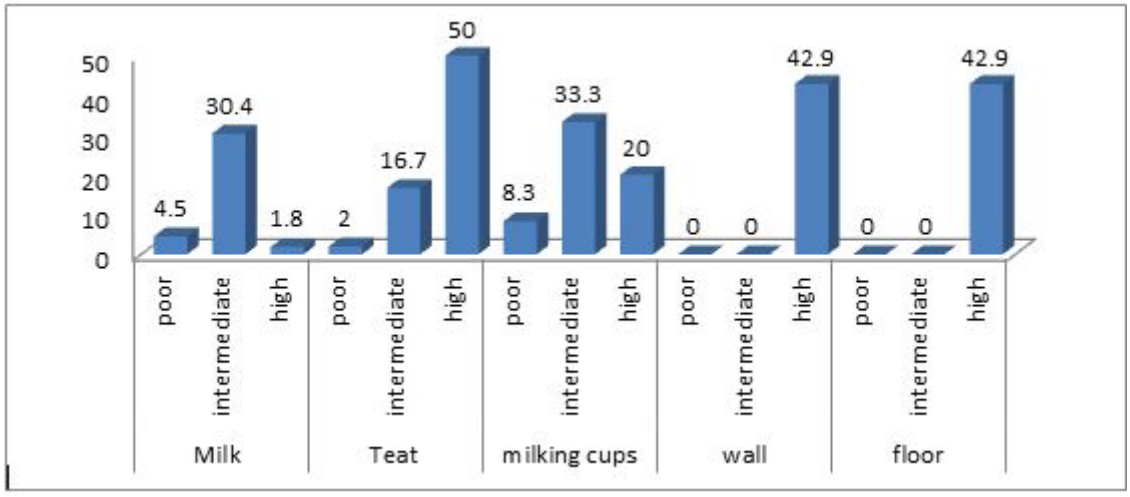

Fig. 3: Prevalence of *E. coli* from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.

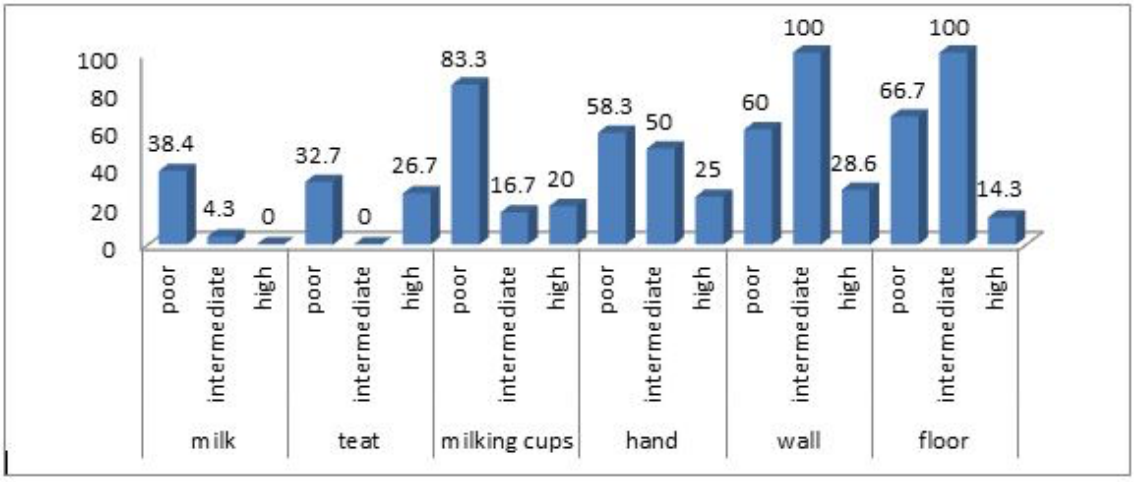

Fig 4: Prevalence of *Citrobacter* from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.

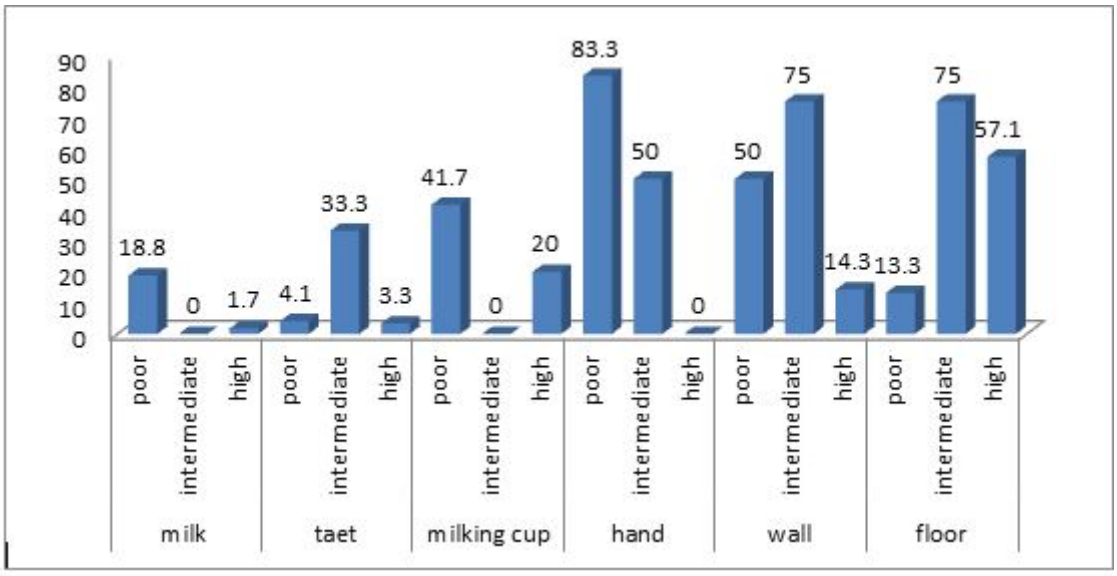

Fig. 5: Prevalence of *Klebsiella* from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.

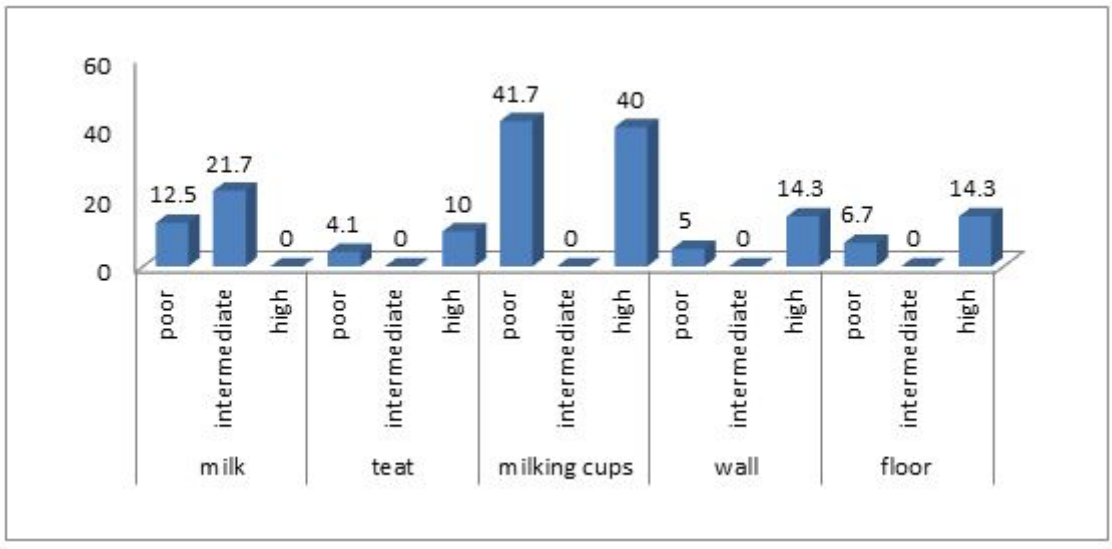

Fig. 6: Prevalence of *Proteus* from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.

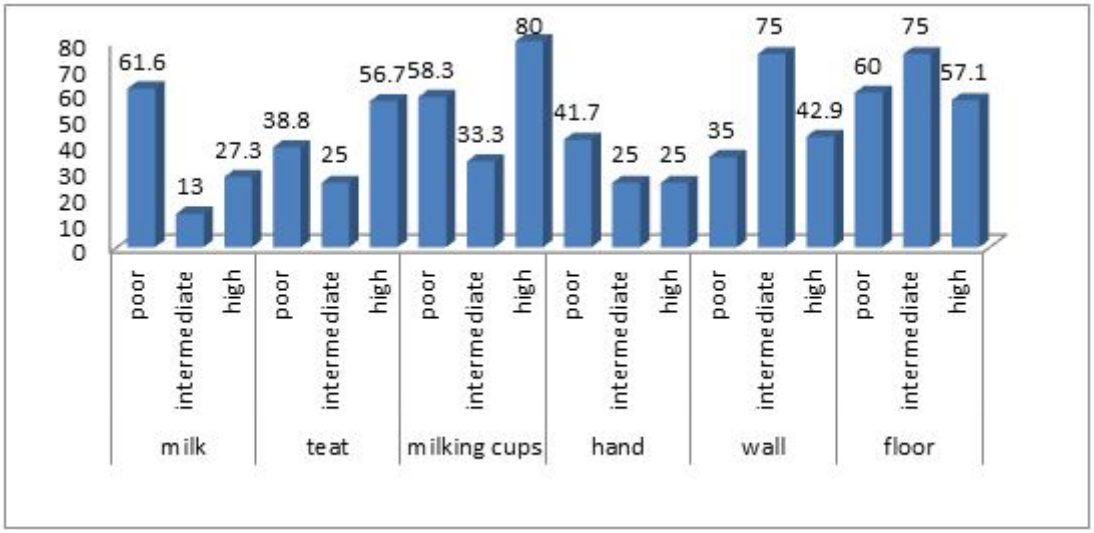

Fig. 7: Prevalence of *Enterococcus* from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.

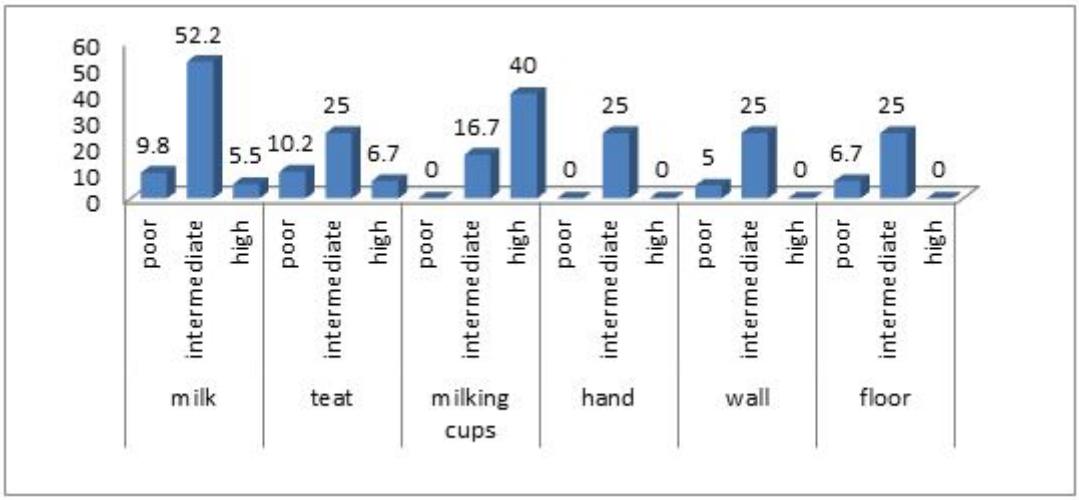

Fig. 8: Prevalence of *Streptococcus dysgalactiae* from milk and environmental samples collected from dairy cattle farms with different biosecurity levels.