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# Prevalence, Associated Risk Factors and Antibiotic Sensitivity Test of *Staphylococcus aureus* and *Escherichia coli* from Bovine Mastitis Milk in and Around Asella Town, Ethiopia

*Dita Abebe, Dechassa Tegegne and Tesfaye Belachew*

College of Agriculture and Veterinary Medicine, School of Veterinary Medicine,
Jimma University, P.O. Box: 307, Jimma, Ethiopia

**Abstract:** Mastitis is one of the most serious problems in dairy industry caused by wide spectrum of pathogens originated from environment or contagious in nature. From these *S. aureus* and *E. coli* play significant economic importance in the dairy industry. Although, mastitis was studied repeatedly but still full epidemiological data is very important particularly in South Eastern part of the country. Therefore, the objectives of this study were to determine the prevalence, associated risk factors and antibiotic sensitivity of *S. aureus* and *E. coli* circulating in dairy farms. A cross sectional study was conducted from November 2017 to March 2018 and around Asella town, south east Ethiopia (n= 384). Clinical examination and California mastitis test were carried out in the field and laboratory culture and primary and secondary biochemical tests were conducted in the laboratory to determine the prevalence and antibiotic sensitivity of *S. aureus* and *E. coli* form bovine mastitis. Logistic regression was used to determine potential risk factors. The overall prevalence of the disease was 48.4%, from this 43.0 and 5.5% mastitis prevalence was found due to *S. aureus* and *E. coli*, respectively. Out of total, 134 lactating local and 250 cross breed cows examined, 186(48.4%) had mastitis, from these 10.7 (41/384) and 37.80% (145/384) showed clinical and subclinical mastitis respectively. Logistic regression analysis indicates that potential risk factors related to lactation stage revealed that the likelihood of *S. aureus* infection was higher in late lactation stage (OR= 4.653(2.646, 8.182, P= 0.000) when compared with early lactation stage. Age was potential risk factor revealed that *E.coli* infection was higher in (>9) age (OR= 5.162 (1.569, 16.987, P= 0.007) when compared with (<6) age. In this study *S. aureus* isolates were most susceptible to Gentamycine, Chloroamphencol, Kanamycin and Vancomycin while resistant to tetracycline and penicillin. Similarly *E. coli* was highly resistant to penicillin and tetracycline but highly susceptible to Gentamycin, Streptomycin, Kenamycin and Chloramphenicol. Therefore, it is particularly a requisite providing awareness about its public health importance and risk factors that expose cows to mastitis infection such as improper washing of udder, poor environment and unclean hand milker's.

**Key words:** Antibiotic Sensitivity Test • California Mastitis Test • *Eschericia coli* • Mastitis • *Staphylococcus aureus*

## INTRODUCTION

Mastitis is the inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis is one of the most complex diseases of dairy cows that generally involves interplay between management practices and infectious agents, having differed causes, degrees of intensity and variations in duration and residual effects [1]. Mastitis is caused by a wide spectrum of pathogens and epidemiologically is categorized into contagious and environmental mastitis [2]. Contagious mastitis Cows suffering from contagious mastitis serve as the major reservoir. They spread from cow to cow, primarily during milking and tend to result in chronic subclinical infection with flare ups of clinical incidence. Contagious pathogens include: Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma spp. and Corynebacterium bovis [3].

**Corresponding Author:** Dita Abebe, College of Agriculture and Veterinary Medicine, School of Veterinary Medicine, Jimma University, P.O. Box: 307, Jimma, Ethiopia.

*Staphylococcus aureus* is a major pathogen of the mammary gland and a common cause of contagious bovine mastitis [4]. Environmental mastitis is the environmental contaminant and cause in acute bovine mastitis [5]. *Escherichia coli* are major environmental contaminant causative agents. The majority of infections caused by environmental pathogens are clinical and of short duration [1] and establishes mild subclinical infection in the udder for a long duration. Bacteria are shed into milk from infected quarters. However, in extreme cases *E.coli* mastitis can lead to severe systemic clinical symptoms like sepsis concurrent with fever [6]. Occasionally, an infection with *E.coli* results in a subclinical and persistent pathology [7].

Mastitis can also be classified as either clinical or subclinical. Clinical mastitis is characterized by sudden onset, alterations of milk composition and appearance, decreased milk production and the presence of the cardinal signs of inflammation in infected mammary quarters [3]. In contrast, no visible signs are seen either on the udder or in the milk in case of sub-clinical mastitis, but the milk production decreases and the somatic cell count increases. It is more common and has serious impact in older lactating animals than in first lactation heifers [8]. Because of the lack of any overt manifestation, the diagnosis of subclinical mastitis is a challenge in dairy animal management and in veterinary practice [3]. Mastitis causes high economic loss in the dairy industries particularly in developing countries like Ethiopia due to reduced milk production and quality deterioration, condemnation of milk due to antibiotic residues, veterinary costs, culling of chronically infected cows and occasional deaths [9]. Moreover, mastitis has a serious zoonotic potential associated with shedding of bacteria and their toxins in the milk [10].

Antimicrobial residues in food animals have many implications on public health; there is a possibility of direct reaction to residues or toxic, anaphylaxis. There is also likely hood of developing drug resistance strains of bacteria. Antibiotics which are frequently and commonly applied in veterinary medicine include: Blactams(penicilin), Aminoglycosides (streptomycin, neomycin), Tetracycline(doxycycline, minocycline), Macrolides (erythromycin and chloramphenicol) [11]. In Ethiopia mastitis has long been known [12]. Although, mastitis was studied repeatedly but still full epidemiological data is very important particularly in South Eastern part of the country in Asella town and around. Therefore, the objectives of this study were to determine the prevalence, associated risk factors and antibiotic sensitivity of *S. aureus* and *E. coli* bovine mastitis in and around Asella town.

## MATERIALS AND METHODS

The study was conducted from November 2017 to March 2018 to determine prevalence, associated risk factors and antibiotic sensitivity testing of *S. aureus* and *E. coli* from bovine mastitis milk in and around Asella town, Arsi zone the Oromia Regional State.

**Study Animals and Husbandry Practices:** The study animals included 384 lactating local and cross breed cows on selected lactating cows regardless of their age, breed, lactation stage and health. Dairy cow are managed under small-scale, extensive and semi-intensive management system.

**Study Design and Sampling Method:** A cross-sectional study type simple random sampling was carried out from November 2017 to March 2018 to determine the prevalence, associated risk factor and antibiotic sensitivity of *Staphylococcus aureus* and *Escherichia coli* from bovine mastitis in and around Asella town. In this study 384 lactating cows were tested for the presence of clinical and sub clinical mastitis. The sampling included all the four quarters of the mammary glands of the cow separately. The milk sample taken were subjected to the CMT to check for mastitis and positive samples for those tests was farther proceeded for further bacteria culture and biochemical tests. The laboratory study was done in Asella Regional Veterinary Laboratory.

**Sample Size Determination:** Simple random sampling strategy was followed to collect milk from the individual animals. To calculate the total size, the sample size was calculated based on the formula described by Thrusfield [13] with 95% confidence interval at 5% desired absolute precision and assumption of the expected prevalence 50%.

$$N = 1.96^2 \times p\,(1-p)/d^2$$

where
N = Sample size
P = expected value
d = desired absolute precision

**Study Methodology**

**Questionaries' Survey:** Data regarding the different potential risk factors (age, parity, lactation stage and breed) was collected for 384 lactating cows from farm records when available and by interviewing the farm owner when not.

**Physical Examination of Udder and Milk:** The udders of the study cows were examined visually and by palpation for presence of clinical mastitis. During examination attention was paid to cardinal signs of inflammation, size and consistency of udder quarters [4]. Inspection of milk performed for discoloration, consistency and presence of clots, which are characteristic of clinical mastitis was done [14].

**California Mastitis Test:** The California mastitis test was used as a screening test for sub-clinical mastitis. It was carried out according to the procedures described by Quinn *et al.* [15].

**Milk Sample Collection and Bacterial Examination:** The milk sample was taken from cows not treated early with either intra mammary or systematic antimicrobials agents. Quarters with CMT one and above were milk sampled during ongoing milking, for further bacterial examination. CMT negative cows (0 and trace scores) would be not included in milk sampling. Milk sampling was carried out following aseptic procedures (Annex III) as described by NMC [16]. After collection, the samples were labeled and placed in icebox and transported to the microbiology of Asella Regional Veterinary Laboratory, Asella. The samples were immediately cultured or stored at 4°C for a maximum of 24hr until cultured on standard bacteriological media. Milk samples were bacteriologically examined according to the procedures involved by Quinn *et al.* [15].

**Antibiotic Sensitivity Testing:** The antibiotic disks were applied on the surface of the inoculated agar plates (Inoculated with Standardizing the bacterial suspension using McFarland standards) using aseptic technique. About seven antimicrobials such as chloroamphenicol, Gentamycine, Penicillin, Streptomycin, Kanamycin, Tetracycline andVancomycine (Oxoid, Hampshire, England) were selected from main class of antimicrobials and investigated for sensitivity testing. Each disk was pressed down to ensure complete contact with the agar surface. After measuring the zone of inhibition, it was classified as sensitive, intermediate and resistant according to National Committee for Clinical Laboratory Standard (NCCLS) break point to interpret the inhibition zone [15].

**Data Management and Analysis:** All raw data that was collected for this study was coded and entered to Microsoft office excel data base system. The findings and data were analyzed by SPSS 20.0 version. Logistic regression was used to see the association of the potential risk factors with occurrence of mastitis using *S. aureus* and *E. coli*. The final model was fit using step wise logistic regression. The degree of association between risk factors and the prevalence of mastitis were analyzed using odds ratio (OR). In all the analysis, the level of significance was set at 5%.

## RESULTS

**Overall Prevalence:** A total of 384 lactating cows was examined for the presence of mastitis both clinically and subclinical by screening with CMT (California Mastitis Test) supported by detailed bacteriological examinations. Of 384 lactating cows examined 213 were positive for CMT, from this 165 *S. aureus* and 21 *E. coli* were isolated. The overall prevalence of the disease was 48.4%. Out of total, 134 lactating local and 250 cross breed cows examined, 186(48.4%) had mastitis, from these 10.7% (41/384) and 37.80% (145/384) showed clinical and subclinical mastitis respectively. In the clinical form there were active cases of mastitis with visible signs of inflammation on the udder, changes in quality and consistency of milk. California mastitis test positive were highly related to *S. aureus (CC= 0.*778 whereas low related to *E. coli* (CC= 0.216) as present in (Table 2).

***S. aureus* Isolation and Associated Risk Factors:** The prevalence of *S.aureus* in the study was 165 (43.0%). *S.aureus* prevalence was higher in early lactation (35.2%) and late stage of lactation (35.8%) but low in mid lactation (29.1%) , the difference is statistically significant ($p<0.05$). Also results of logistic regression analysis indicates that potential risk factors related to lactation stage revealed that the likelihood of *S.aureus* infection was higher in late lactation stage (OR= 4.653, CI= 2.646, 8.182, p= 0.000) when compared with early lactation stage (Table 3).

*S. aureus* prevalence showed no significant variation among different age groups (≤5 (33.9%,), 6-9 (37.5%, P=0.293)) and ≥9 (28.5%, P=0.078), parity (≤3 (35.2%, P= 0.535), 3-4 (51.9%, P=0.305)), ≥5(30.3%, p=0.840) and breed (Local (31.5%, p=and cross (68.5%, p=0.111)) (Table 3).

Table 1: Correlation Coefficient of California mastitis test, *S. aureus* and *E. coli and* their prevalence in clinical and subclinical mastitis

| Form | No. cow examined | *S. aureus* (+) | *E. coli* (+) | Total |
|---|---|---|---|---|
| Clinical | 52 | 34 | 7 | 41(78.8%) |
| Subclinical | 332 | 131 | 14 | 145(43.7%) |
| CMT positive | 213 | 165(43.0) | 21(5.5%) | 186(87.3) |
| CMT SCORE CC | - | - | $0.216^{**}$ | - |
| p. value | | 0.000 | 0.000 | - |

CC= Correlation Coefficient, ** Correlation is significant at the 0.01level (2-sided tail)

Table 2: Logistic regression analysis of the association of different potential risk factors associated with *S. aureus*

| | | | Univariable | | Multivariable | |
|---|---|---|---|---|---|---|
| Risk factors | Total | No of positive | COR (95% CI) | P. value | AOR(95% CI) | P. value |
| Breed | | | | | | |
| Local | 134 | 52( 31.5%) | 1 | | - | - |
| Cross | 250 | 113( 68.5%) | 1.449 (0.918, 2.286) | 0.111 | - | - |
| Age | | | | | | |
| ≤5 | 134 | 56(33.9%) | 1 | | | |
| 6-9 | 163 | 62(37.6%) | 1.379(0.757, 2.512) | 0.293 | - | - |
| ≥9 | 87 | 47(28.5%) | 1.67 5 (0.944, 2.970) | 0.078 | - | - |
| Parity | | | | | | |
| ≤3 | 145 | 58(35.2%) | | | | |
| 3-5 | 135 | 57(51.9%) | 1.345(0.764, 2.369) | 0.305 | - | - |
| ≥5 | 104 | 50(30.3%) | 1.060(0.601, 1.869) | 0.840 | - | - |
| Lactation stage | | | | | | |
| Early | 143 | 58(35.2%) | 1 | | - | - |
| Medium | 154 | 48(29.1%) | 3.077(1.738, 5.447) | 0.000 | 3.088(1.764, 5.407) | 0.000 |
| Late | 87 | 59(35.8%) | 4.582(2.578, 8.142) | 0.000 | 4.653(2.646, 8.182) | 0.000 |

Table 3: Logistic regression analysis of the association of different potential risk factors associated with *E. coli*

| | | | Univariable | | Multivariable | |
|---|---|---|---|---|---|---|
| Risk factors | Total | No of positive | COR (95% CI) | P. value | AOR (95% CI) | P. value |
| Breed | | | | | | |
| Local | 134 | 9 (42.9% ) | 1 | | - | - |
| Cross | 250 | 12(57.1% | 0.702(0.280, 1.758) | 0.450 | - | - |
| Age | | | | | | |
| ≤6 | 134 | 7(33.3%) | 1 | 0.024 | | 0.020 |
| 6-9 | 163 | 4(19.0%) | 1.925 (0.639, 5.796) | 0.244 | 2.356(.861, 6.447) | 0.095 |
| ≥9 | 87 | 10(47.6%) | 5.716(1.633, 20.00) | .006 | 5.162(1.569, 16.987) | 0.007 |
| Parity | | | | | | |
| ≤3 | 145 | 5(23.8%) | 1 | | | |
| 3-5 | 135 | 7(33.3% ) | 2.437(0.720, 8.253) | 0.152 | - | - |
| ≥5 | 104 | 9(42.9%) | 1.177(0.387, 3.578) | 0.774 | - | - |
| Lactation stage | | | | | | |
| Early | 143 | 8(38.1%) | 1 | | | |
| Medium | 154 | 10(47.6%) | 0.488(0.123, 1.943) | 0.309 | - | - |
| Late | 87 | 3(14.3%) | 0.395(0.102, 1.528) | 0.178 | - | - |

Key: COR= crude odd ratio, AOR= adjusted odd ratio, CI= Confidence Interval

***E. coli* Isolation and Associated Risk Factors:** From 384 lactating cows examined 213 were positive for California mastitis test. Out of positive CMT score, 21(5.5%) *E. coli* was isolated in the study. It was taken into consideration and the occurrence of mastitis was measured for different age groups of lactating cows. *E.coli* prevalence showed significant variation among age groups (≤5 (33.3%), 5-9 (19.0%) and ≥9 (47.6%)) were statistically significant at ($p<0.05$). Results of logistic regression analysisalso indicates that potential risk factors related to age revealed that *E. coli* infection was higher in (≥9) age (OR= 5.162, CI=1.569, 16.987, P= 0.007) when compared with (≤5) age

Table 4: Antibiotic Sensitivity test for *S. aureus* (N = 16) and *E. coli* (N = 12)

| | | *S. aureus* | | | *E. coli* | | |
|---|---|---|---|---|---|---|---|
| Antibiotics | Unit (μg) | S (%) | I(%) | R (%) | S (%) | I (%) | R (%) |
| Gentamycine | 10 | 14(87.5) | - | 2(12.5) | 12(100) | - | - |
| Chloroamphencol | 30 | 16(100) | - | - | 6(50) | - | 6(50) |
| Vancomycin | 30 | 16(100) | - | - | - | - | 12(100) |
| Streptomycin | 10 | 3(18.75) | 3(18.75) | 10(62.5) | 12(100) | - | |
| Kanamycin | 30 | 16(100) | | | 6(50) | - | 6(50) |
| Penicillin | 10 | - | - | 16(100) | - | - | 12(100) |
| Tetracycline | 30 | - | - | 16(100) | - | 3(25) | 9(75) |

Keys: N = Number of observation, S = Susceptible, I = Intermediate, R = Resistance

(Table 4). *E. coli* prevalence showed no significant variation among different parity (<3 (23.8 %, ), 3 4 (33.3%, P= 0.152), >5(42.9%, P=0.774), lactation stage (early 38.1%, medium 47.6%, P= 0.309 and late 14.3%, p= 0.178) and breed (Local 42.9 and cross 57.1%) (Table 3). Breed, age and lactation stage were statistically insignificant (p>0.05).

**Antimicrobial Susceptibility Test:** From total positive samples, sixteen and twelvee were tested for susceptibility of *S.aureus* and *E.coli* to different seven antimicrobial discs. In the current study *S. aureus* isolates were sensitive to Vancomycin (100%), Chloroamphencol (100%), Kanamycin (100%) and Gentamycine (87.5%). streptomycin (18.75%), whereas intermediate to streptomycin (18.75%) and resistant to Tetracycline, Penicillin and streptomycin with the isolates having sensitivity of 100, 100 and 62.5% respectively.

*E. coli* isolates were sensitive to Gentamycine (100%), streptomycin (100%), Chloroamphencole (50%) and Kanamycine (50%) whereas intermediate to Tetracycline (25%) and resistant to Vancomycin, Penicillin and Tetracycline with the isolates having sensitivity of 100, 100 and 75%, respectively.

## DISCUSSION

Mastitis prevalence in this study due to *S. aureus* and *E. coli* was 48.4% in agreement with Kero and Tarek [17], Abdelrahim *et al.* [18] and Radostits *et al.* [19], who reported (40, 45.8 and 50%) respectively. However, the present findings are lower than the prevalence's mastitis reported (52.8% around Sebeta, 53.5% in Kallu province, 61.11% in South Wollo, 66.6% in Asella and 68.1% in Addis Ababa with Hundera *et al.* [20], Tolosa *et al.* [21], Tolla [22], [12] and Zerihun [23], respectively. The studies showed lower prevalence 33.0% and 27.3%) in Wolaita with Tolosa *et al.* [21] and Biffa [24] respectively. This variability in prevalence of mastitis between different reports could be attributed to differences in farm management practices or to differences in study methods and test employed by the researcher (Refs.??).

With regard to the bacteriological analysis of milk samples, the work revealed that from 213 CMT positive milk samples 131 and 34 *S. aureus* were isolates from subclinical and clinical, which were higher than 14 and 7 *E. coli* isolates from subclinical and clinical mastitis respectively. This might be due sample collection *S. aureus* infection at early stage and *E. coli* infection at late lactation stage. At this phase, infection with *S. aureus* increases and *E. coli* infection decreases.

In this study prevalence of sub-clinical mastitis (37.8% and clinical mastitis (10.7%) agrees with Kero and Tarek [17], Hundera *et al.* [20] and Mekibib *et al.* [25], who reported (48.6% versus 22.4%) in Holeta, (62.9 versus 37.0% in Southern Ethiopia), (36.67 versus 16.11%) in central, Ethiopia). The variability in the prevalence of bovine mastitis between reports could be attributing to the difference in management of the farm, breeds, season of the study, agro climactic condition or diagnostic test employed.

The species of bacteria isolated *S. aureus* was most commonly isolated in clinical and sub clinical cases of mastitis in this study case. The high level isolation of *S. aureus* (43.0%) in this study is related with the finding of Mekibib *et al.* [25] in Holeta who reported 47.1 %, but lower than Ahmed and Mohammed [26] in Egypt who reported 52.5% and higher than Bedada and Hiko [27] in Asella who reported 39.1%. The reason for the higher isolation rate of this organism is the wide ecological distribution inside the mammary gland and skin. In area where hand milking and improper use of drug is practiced to treat the mastitis cases, its domination has been reported by many research scholars. *S. aureus* is adapted to survive in the udder and usually establishes mild sub clinical infection of long duration from which it is shaded through milk serving as sources of infection for other healthy cows transmitted during the milking process [28]. Hence, the organism has been assuming a position of major importance as a cause of bovine mastitis.

The 5.5% isolation of *E.coli* found in this study is comparable with the findings of Mekibib *et al.* [25] who reported 4.6%. Isolated *E.coli* found in this study is higher than the findings of Bedada and Hiko [27] in Asella, Mekonnen *et al.* [29] in Ethiopia Dairy and Ashenafi [30] in Kombolcha, who reported (2.2, 3.9 and 3.9%), respectively. In contrast, *E. coli* in the study was lower than Sumathi *et al.* [31], who reported 20%. The prevalence of environmental *E.coli* may be associated with poor farm cleanliness and poor slope of stable areas. Feces which are common sources of *E. coli* can contaminate the premium directly or indirectly through bedding, calving stalls, udder wash water and milker's hands [4].

Results of logistic regression analysis indicates that potential risk factors related to lactation stage revealed that the likelihood of *S.aureus* infection was higher in late lactation stage (OR= 4.653, 95% CI2.646, 8.182) in comparison to cows at the early lactation stage. The present investigation is in line with the reports by Abera *et al.* [32] late lactation stage (OR = 3.2, 95% CI = 1.2, 8.2) higher than early lactation stage. Radostits *et al.* [19] suggested that, the mammary gland is more susceptible to new infection during the early and late dry period, which may be due to the absence of udder washing and teat dipping, which in turn might have increased the presence of potential pathogens on the skin of the teat.

The finding of this study was assessed for breed predisposition to mastitis due to *S. aureus and E. coli,* but no significant difference in the prevalence was detected between the two breeds. It has been reported that mastitis prevalence may be influenced by some inheritable characteristic such as capacity of milk production, teat characteristic and udder conformation [33].

However, the insignificant difference in the prevalence of mastitis between the two breeds reported in this work needs further investigation before a satisfactory explanation is being forwarded.

*E. coli* occurrence showed significant variations among age groups ($\leq$5 (33.3%), 5-9 (19.0%) and $\geq$9 (47.6%) which were statistically significant at ($p<0.05$). Results of logistic regression analysis indicates that potential risk factors related to age revealed that *E.coli* infection was higher in ($\geq$9) age (OR= 5.162(1.569, 16.987) when compared with ($\leq$5) age. This study is comparable with the findings of Mahmoud *et al.* [34] in Behira governorate. Evidence from earlier studies has shown that young cows are more resistant than older cows due to their more alert defence mechanisms [35]. The increased prevalence of *E. coli* in older animals in this study can be related to increased susceptibility of pathogenic organisms in udder relaxed sphincter muscles of teats.

In this study *S. aureus* isolates were most susceptible to Gentamycine, Chloroamphencol, Kanamycin and Vancomycin while resistant to tetracycline and penicillin. Similarly *E. coli* was highly resistant to penicillin and tetracyclin but highly susceptible to Gentamycin, Streptomycin and Kenamycin and. These were comparable with the findings of Mekonnen *et al.* [29] and Abera *et al.* [32].

## CONCULSIONS

The present study showed that the overall prevalence of mastitis due to *S. aureus* and *E. coli* was (48.4%). In this study lactation stage and age of cows were risk factor associated significantly with the mastitis caused by *S.aureus* and *E.coli*, respectively. The risk factors related to age, breed, parity and lactation stage also showed some prevalence of *S. aureus* (except first) and *E. coli* (except last factor), but statistically insignificant. In this study *S.aureus* isolates were most susceptible to Gentamycine, Chloroamphencol, Kanamycin and Vancomycine while resistant to tetracycline and penicillin. Similarly *E.coli* was highly resistant to penicillin and Tetracyclin but highly susceptible to Gentamycin, Streptomycin, Kenamycin and Chloramphenicol. Therefore, it is particularly requisite providing awareness about its public health importance and risk factors that expose to cows the mastitis infection such as improper washing of udder, poor environment and unclean hand milker's.

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**Authors' Contributions:** DA conceived and designed the study protocol. DA and TB carried out sample collection and laboratory examination. DA interpreted the results of data analysis and drafted the manuscript and also compiled the results, improved and corrected the manuscript. All authors read, commented and approved the final manuscript.

**Competing Interest:** The authors declare that they have no competing interests.

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