Bovine mastitis: Prevalence, causes and associated risk factors in Silte Zone, Ethiopia

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Abstract

Mastitis is an important disease of dairy cows, and it causes huge economic losses to dairy farm owners due to a decrease in milk production, and reduction in milk quality, and an increase in the cost of cow treatment. A cross-sectional study was conducted in Southern Ethiopia's Silte zone from October 2020 to June 2021 aimed to estimate mastitis prevalence, assess related risk factors, and identify prevalent bacterial causes. Three hundred eighty-four lactating cows were examined for abnormalities in udder quarters and teats. Milk samples were tested for subclinical mastitis via the California mastitis test (CMT) and cultured for causative agents from clinical mastitic and CMT-positive cows. The overall prevalence of mastitis was 54.9% (95% CI=49.9-59.9), of which 52.1% (95% CI=47.1-57.1) was subclinical and 2.9% (95% CI=1.6 -5.1) clinical mastitis. From a total of 1536 quarters examined 41 (2.7%) quarters were found blind and 427 (27.7%) quarters were affected by mastitis. Overall, more hind quarters, 236 (55.5%) were affected than the front quarters, 189 (44.5%) of udder. The prevalence of mastitis was significantly higher during early lactation, ≤4 months ($p < 0.05$); and it was increased with increasing parity ($p < 0.05$) and age ($p < 0.05$). Cows with no bedding were more affected than those with bedding ($p < 0.05$). Multivariable logistic regression showed that cows with round and flat teat ends were 2.84 and 11.85 times more likely to contract mastitis. Also, cows with pendulous udder, producing more than 10 liters per day milk and during the early 4 months of lactation were 1.87, 6.81, and 2.14 more likely affected by mastitis than normal udder, producing less than 10 liters of milk per day and lactation after five months, respectively. Milk samples collected from 211 mastitis-positive cows were cultured using standard bacteriological technique, and the isolated bacteria were *Staphylococcus aureus* (29.5%), *Staphylococcus epidermidis* (14.2%), *Staphylococcus intermedius* (11.6%), *Staphylococcus hyicus* (11.1%), *Streptococcus agalactiae*

(8.9%), *Streptococcus disgalactiae* (6.3%), *E. coli* (5.8%), *Streptococcus uberis* (5.3%), *Klebsiella* spp. (1.6%) and *Enterococcus* spp. (1.1%). Owners should give proper udder care and are advised to apply dry cow therapy. Extension workers should raise awareness.

Keywords: Mastitis; Prevalence; risk factor; Silte zone; Ethiopia.

Introduction

Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause. It is characterized by a range of physical and chemical changes in the milk and pathologic changes in the glandular tissue (Constable *et al.*, 2017). Bovine mastitis is a multifactorial inflammatory disease that depends on a combination of animal, environmental, and pathogen-related factors (Hillerton and Berry, 2005).

Mastitis is one of the most prevalent and costly diseases in dairy production with losses attributable to milk production losses, milk quality, discarded milk, early culling, veterinary services, and labor costs (Seegers *et al.*, 2003; Hogeveen, and *Østerås*, 2005). Mastitis has two forms: clinical and subclinical. The major causes of bovine mastitis are classified into contagious pathogens, for example, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *M. bovis*) and environmental pathogens, for example, Streptococcus uberis, Streptococcus dysgalactiae, E. coli, *Klebsiella* spp., and *Enterobacter* spp.) (Constable *et al.*, 2017).

In both clinical and subclinical mastitis, there is a loss in milk production. Moreover, the loss in milk production does not only occur during the case itself, even after the mastitis case is cured, the milk production level of the cow stays lower. Milk production loss is not obvious to the producer, because this is milk never produced, and therefore never seen. It is a hidden cost or lost income opportunity (Hogeveen, and *Østerås*, 2005). Moreover, milk from mastitic cows is potentially a risk to public health, because it may transmit zoonoses and sicknesses associated with food toxins (Zouharva and Rysanek, 2008; FAO, 2014). *Staphylococcus aureus* produces staphylococcal enterotoxins that may cause food poisoning, which is characterized by acute onset of nausea, vomiting, abdominal cramps, and diarrhea (Le Loir *et al.*, 2003; Zouharva and Rysanek, 2008).

In Ethiopia, the prevalence of mastitis ranged from 25.1% to 73.7% (Fesseha *et al.*, 2021; Girma and Tamir, 2022). The higher prevalence of mastitis in lactating cows is greatly influencing the productivity of the animals. The potential predisposing factors so far studied include breed, parity, age of cow, lactation stage, bedding, udder position, teat end shape and morphology, and other management factors like feeding and hygiene (Getaneh and Gebremedhin, 2017; Kitila *et al.*, 2021; Girma and Tamir, 2022).

There is no information about bovine mastitis from the Silte zone; thus, the study gives some information about mastitis in selected districts of the Silte zone. So, this study is useful to add to the existing knowledge of mastitis status in the country. Therefore, the purpose of this study is to estimate the prevalence of bovine mastitis, to identify the major bacterial causes circulating in the area, and to assess the potential predisposing risk factors in the study area.

Materials and methods

Study areas

The study was conducted from October 2020 to June 2021 in selected districts of the Silte zone, Southern Ethiopia. The zone is located at 7.43^0 N to 8.1^0 N latitudes and 37.86^0 E to 38.53^0 E longitudes. The area is generally characterized by diverse agro-climatic conditions with altitudes ranging from 1500 meters to 2,100 masl. The mean annual temperature and annual rainfall range from 15.1°C to 27.5°C and 800 to 2,000 mm, respectively (SZLFRD, 2020).

Study animals and study design

During the informed consent for cow udder examination and milk sample collection as well as the willingness to participate in the study was obtained from the animal owners. The study animal includes lactating cows (local zebu, and Holstein-cross breeds), that are found in and around Worabe. They were kept under small-scale and semi-intensive management systems. All the study cows were hand milked and milked twice a day, and the study included small-holder (i.e. having 1-10 lactating cows) and large-scale (i.e. having ≥10 lactating cows) dairy farms.

A cross-sectional study design was applied to estimate the prevalence of mastitis in lactating dairy cows and to investigate the major bacterial causes of mastitis in the study areas. Also, the risk factors contributing to the development of mastitis were assessed. The study was conducted from October 2020 to June 2021. The potential risk factors considered for this study were teat end shape, udder position, milk yield per day, parity of cow, lactation days, breed, age, parity, bedding material, and district. Teat end shape and udder position were assessed as described by Bakken (1981) and the age of cows was determined by using dentition (Parish and Karisch, 2013). But information about milk yield per day, parity, and lactation days was collected by direct interviews of the owners during farm visits for the selection of the study cows and milk sampling.

Sample size and sampling methods

The study areas: Worabe town and two surrounding districts (Dalocha and Hulbarag) were selected because of their dairy production potential. In this study, lactating cows were selected by using a systematic random sampling technique. The sample size was determined by considering 50% expected prevalence (i.e. to have the maximum sample size), 95% confidence level, and 5% desired level of precision (Thrusfield, 2018). Hence, the total sample size required for the study was 384 lactating cows. The computed sample was proportionally allocated to the three study areas based on the number of dairy cows present in each area (Table 1).

Table 1. Proportional allocation of the number of study animals

District	Number of Lactating Cows	Sample size
Worabe	4202	138
Dalocha	3957	130
Hulbarag	3531	116
Total	11690	384

Source: Dairy cows data (SZLFRD, 2020).

Study methodology

Clinical examination of cows and milk

All quarters of the udders and teats of selected lactating cows were examined visually and by palpation for the presence of the manifestation of general clinical signs like a gross abnormality, inflammatory swelling and pain, hardness, atrophy of tissue, and teat blindness. The milk sample was checked for the presence of clots, flakes, and blood by using a strip cup (Andrews *et al*., 2004; Quinn *et al.,* 2004).

Screening test (CMT)

All udder quarters irrespective of the physical, clinical, and examination results were tested by using California Mastitis Test (CMT) to investigate the affected quarter. During this time teat area was cleaned by using 70% denatured alcohol. After the first few squirts of milk were discarded, about 2ml of milk sample (i.e. approximately ½ teaspoon) were collected into a CMT paddle with four shallow cups. An equal amount of CMT reagent was added to each milk sample. Then after, the paddle was rotated to mix the contents. The CMT result was interpreted as negative=0, weak positive=+1, positive=+2, and strongly positive=+3 (Quinn *et al*., 2004; Constable *et al*., 2017). A cow was considered positive when at least one-quarter was positive for CMT.

Milk sample collection and culture

For identification of the causative agent/s milk samples were collected from all cows with clinical mastitis and CMT-positive cows. Udder and teat were cleaned thoroughly and dried with a clean towel, then, each teat end was cleaned with 70% ethyl alcohol. The first few streams of milk were discarded and approximately 10 ml of milk was aseptically collected from each quarter of lactating cows into a sterile test tube. Each test tube was labeled with the necessary information for identification. The samples were immediately transported by the ice box (4°C) to Sodo Regional Veterinary Laboratory, Wolaita Sodo town. Collected milk samples were subjected to bacteriological examination by following the standard procedure described in Quinn *et al*. (2004), and then it was immediately cultured. A loop full of milk samples was streaked on blood agar base enriched with 7% sheep blood and MacConkey agar, and incubated aerobically at 37ºC for 24 to 48 hours. Then, the plates were examined for growth, morphology, and hemolytic characteristics of colonies. Colonies were identified based on their morphology, color and size, presence or absence of hemolysis, and gram stain (Quinn *et al*., 2004). Gram stain reaction including shape and arrangements of the bacteria, catalase, and O-F tests. And then, subcultures were done to obtain pure isolate for further identification. For further identification, biochemical tests were conducted by following the standard methods described by Quinn *et al*. (2004) and Carter and Cole (1990). Gram-positive bacteria were further identified into genera and species based on catalase and oxidase test, tube coagulase test; and ability to produce hemolysis and ferment maltose and growth on mannitol salt agar. Gram-negative bacteria were identified based on the growth characteristics on MacConkey agar, as well as Oxidase reaction, catalase test, triple sugar iron agar, and "IMViC" test.

Data analysis

All collected data were entered into a Microsoft Excel spreadsheet, edited, coded, and then summarized by descriptive statistics like percentages and mean. For the data analysis, STATA 14.2 software (Stata Corp 4905 Lakeway Drive, College Station, Texas 77845 USA) was used. The prevalence was computed by dividing the number of mastitis-positive cows by the total number of examined cows and multiplying by 100. The association of the potential risk factors and the prevalence of mastitis was analyzed by univariable logistic regression analyses followed by multivariable logistic regression analysis. Then, after multicollinearity testing, all non-collinear variables with $p < 0.25$ were subjected to multivariable logistic regression analysis. Then, the model fitness was assessed by the Hosmer–Lemeshow goodness-of-fit test (Dohoo *et al*., 2009).

Results

Animal-level prevalence of mastitis

From a total of 384 cows examined both clinically and by CMT for subclinical 211 (54.9%, 95% CI=49.9-59.9) of them were found affected by clinical and subclinical mastitis (Table 2). The prevalence of subclinical mastitis was significantly (χ^2=12.31, $p < 0.05$) higher than that of clinical mastitis.

Table 2. The overall prevalence of mastitis in lactating cows in selected districts of the Silte zone

Study areas	No. Examined	Subclinical Mastitis		Clinical Mastitis		Overall	
		No Pos.	Prevalence (95% CI)	No Pos.	Prevalence (95% CI)	No Pos.	Prevalence (95% CI)
Worabe	138	72	52.2 (43.7-60.5)	3	2.2 (0.7-6.6)	75	54.3 (45.9-62.6)
Dalocha	130	66	50.8 (42.1-59.4)	6	4.6 (2.1-10.0)	72	56.2 (47.4-64.5)
Hulbarag	116	62	53.4 (44.2-62.4)	2	1.7 (0.4-6.8)	64	54.3 (45.1-63.3)
Total	384	200	52.1 (47.1-57.1)	11	2.9 (1.6 -5.1)	211	54.9 (49.9-59.9)

No. Pos. = number positive

Quarter-level prevalence of mastitis

From a total of 1536 quarters examined 41 quarters were found blind (i.e. 39 cows were with a blind quarter of which thirty-seven and two cows with one and two-quarters blind, respectively). So, the remaining 1495 quarters were examined and 425 (28.4%) of them were found positive for mastitis. Of the total positive quarters the subclinical mastitis accounted for 56.7% (n=241) and clinical mastitis for 46.3% (n=184). Relatively the right hind quarters were more frequently affected (55.5%) than the left hind quarters (44.5%) (Table 3).

Table 3. Prevalence of mastitis at the quarter level

Quarter sampled	Total examined	Positive for mastitis		Blind No. (%)
		Number positive (%)	95% CI	
Right front	384	95 (24.7)	20.7-29.3	3 (0.8)
Right hind	384	128 (33.3)	28.8-38.2	2 (2.1)
Left front	384	94 (24.5)	20.4-29.1	6 (4.2)
Left hind	384	108 (28.1)	23.8-32.9	14 (3.6)
Total	1536	425 (27.7)		41 (2.7)

Bacterial isolation

For causative bacterial agents isolation milk samples were collected from 211 mastitic cows (i.e. clinical and subclinical mastitis) and cultured. From these samples (n=211) about 21(9.95%) showed no growth and in 190 (90.05%) of them there was bacterial growth. In culture-positive samples, both contagious and environmental bacteria were isolated, and a total of eleven bacterial species were identified. The most commonly identified bacteria were *Staphylococcus* spp. (n=126, 66.3%), followed by *Streptococcus* spp. (n=39, 20.5%).

The most commonly isolated bacterial spp. was coagulase-positive staphylococci [*Staphylococcus aureus* (29.5%), *Staphylococcus intermedius* (11.6%), and *Staphylococcus hyic- us* (11.1%)] which is followed by coagulase-negative staphylococci [*Staphylococcus epidermidis* (14.2%)]. The proportion of causative bacterial agents isolated during this study is shown in Figure 1.

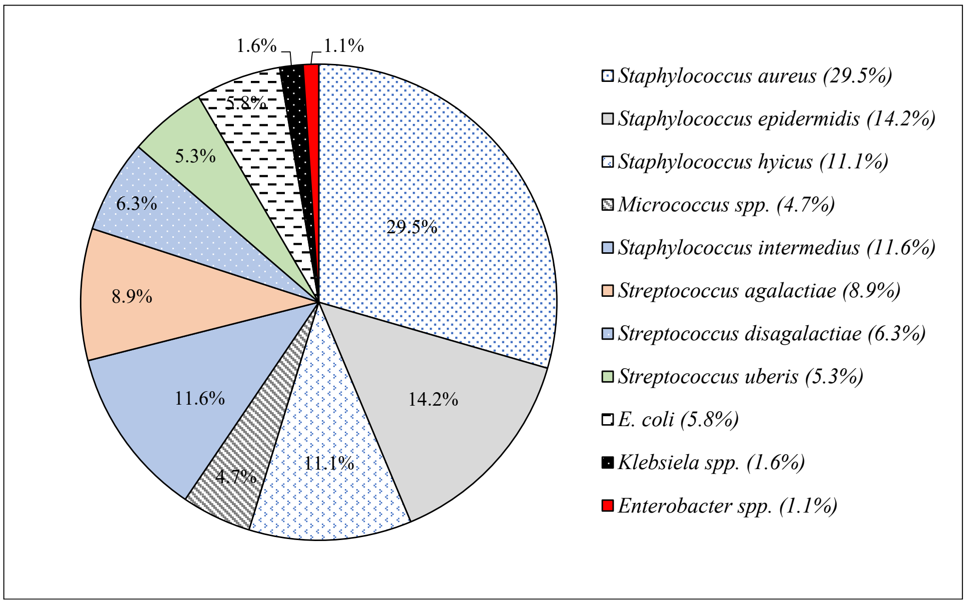

Figure 1. Proportion of bacterial species isolated from mastitic lactating cows

Risk factors analysis

A univariable logistic regression analysis revealed that the occurrence of mastitis was significantly ($p<0.05$) associated with parity, lactation days, age, the shape of the teat end, udder position, amount of milk yield, and bedding materials (Table 4).

The multicollinearity matrix revealed that the age of the cow and parity are collinear to each other ($\Gamma = 0.97$). Similarly, udder position and parity are collinear to each other (Γ = 0.83), and udder position and age are collinear to each other (Γ = 0.79). Biologically it is plausible to drop age and parity from the multivariable logistic regression analysis and consider the udder position that is influenced by age and calving numbers of the cow. So, the stage of lactation, teat end shape, milk yield per day, udder position, and bedding was selected for the final multivariable regression model (Table 4). The multivariate logistic regression model has Hosmer-Lemeshow $\chi^2(7) = 13.26$, p=0.066, ROC = 0.839, and this suggests that there is no significant difference between the observed and predicted values. The sensitivity, specificity, and positive and negative predictive values of the models are 77.25%, 74.57%, 78.74%, and 72.88%, respectively.

Table 4. Univariable logistic regression analysis of the association of mastitis risk factors considered for the study area

Risk factors	Total	Infected (%)	95% CI	Univariable			Multivariable		
				OR	95% CI	P-value	OR	95% CI	P-value
Teat end shape									
Pointed	146	50 (34.25)	27.0-42.4	Ref	-	-	Ref	-	-
Round	133	70 (52.63)	44.1-61.0	2.1	1.31-3.46	0.002	2.84	1.60-5.04	0.000
Flat	105	91 (86.67)	78.7-92.0	12.5	6.46-24.11	0.000	11.85	5.48-25.62	0.000
Udder position									
Normal	237	107 (45.15)	38.9-51.6	Ref			Ref	-	-
Pendulous	147	104 (70.75)	62.8-77.6	2.9	1.90-4.55	0.000	1.87	1.07-3.29	0.029
Milk yield per day									
<10 liter	155	47 (30.3)	23.6-38.1	Ref					
≥10 liter	229	164 (71.6)	65.4-77.1	5.8	3.71-9.07	0.000	6.81	3.95-11.73	0.000
Age (Years)									
3–5	145	67 (46.21)	38.2-54.4	Ref					

Risk factors	Total	Infected (%)	95% CI	Univariable			Multivariable		
				OR	95% CI	P-value	OR	95% CI	P-value
6–9	148	91 (61.49)	53.4-69.0	1.86	1.17-2.96	0.009			
≥10	91	53 (58.24)	47.8-68.0	1.62	0.96-2.76	0.073			
Lactation days									
≤ 120 days	250	162 (64.8)	58.6-70.5	3.20	2.06-4.94	0.000	2.14	1.25-3.67	0.006
≥ 121 days	134	49 (36.6)	28.8-45.1	Ref			Ref	-	-
Parity									
1-2	145	66 (45.5)	37.5-53,7	Ref					
3-4	155	92 (59.4)	51.4-66.8	1.75	1.11-2.76	0.017			
≥5	84	53 (63.1)	52.2-72.8	2.05	1.18-3.55	0.012			
Bedding material									
Yes	254	150 (59.1)	52.9-65.0	1.63	1.07-2.50	0.024	1.38	0.80-2.39	0.245
No	130	61 (46.9)	38.5-55.6	Ref					
District									
Worabe town	138	75 (54.3)	45.9-62.6	Ref					
Dalocha	130	72 (56.2)	47.4-64.5	1.08	0.66-1.74	0.77			
Hulbarag	116	64 (54.3)	45.1-63.3	1.0	0.61-1.64	1.00			

Discussion

The current study showed that the overall prevalence of mastitis in lactating cows was 54.9% of which the clinical and subclinical mastitis accounted for 2.9% and 52.1%, respectively. This study finding is relatively comparable with various reports from different parts of the country (Sori *et al*., 2005; Lakew *et al*., 2009; Abunna *et al*., 2013; Teklesilasie *et al*., 2014; Pal *et al*., 2017; Abebe *et al*., 2020). Subclinical mastitis was more prevalent (54.9% and 95% CI= 49.9-

59.9) than clinical mastitis (2.9% and 95% CI= 1.6 -5.1) in the study area, which is in agreement with various studies reported from various parts of the country (Getaneh and Gebremedhin, 2017; Abebe *et al*., 2020; Fesseha *et al*., 2021; Belay *et al*., 2022; Demil *et al*., 2022; Girma and Tamir, 2022). Subclinical mastitis causes huge economic losses (Azooz *et al*., 2020; Singh *et al*., 2021), which is mostly not recognized timely by owners. According to Sinha *et al*. (2014), the economic loss due to a reduction in milk yield was estimated to be 49%.

A relatively higher proportion of mastitis was observed in the hind quarter of cows, which is similar to previous study reports (Abera *et al*., 2012; Zenebe *et al*., 2014; Abebe *et al*., 2016; Zeryehun and Abera, 2017). The hind quarters are characterized by higher milk yield and longer time of milking (Tacin *et al*., 2006), and these factors are potential indicators for the development of mastitis and high somatic cell count (Kokca, 2006).

The multivariable logistic regression analysis showed that among the potential risk factors considered for this study lactation days, milk yield per day, udder position, and teat end shape were significantly influencing the prevalence of mastitis. This finding is in general agreement with Sori *et al*. (2005), Zenebe *et al*. (2014), and Abebe *et al*. (2016). The occurrence of mastitis is significantly ($p < 0.05$) higher during the early lactation period (Zenebe *et al*., 2014; Getaneh and Gebremedhin, 2017; Zeryehun and Abera, 2017), especially with environmental pathogens (Constable *et al*., 2017). This might be due to the absence of a dry cow therapy regime could be the major contributing factor to a high prevalence of mastitis at early lactation. Higher milk yielding and large teat canal diameter were associated with an increased risk of intramammary infection (Sori *et al*., 2005; Constable *et al*., 2017). There is a genetic correlation between milk yield and mastitis, and this correlation suggests that genes that increase milk yield tend to increase susceptibility to mastitis (Constable *et al*., 2017).

The prevalence of mastitis was significantly higher in round and flat shape teat ends ($p < 0.05$), which is in accord with the reports of Bakken (1981) and Hickman (1964). The teat apex is the first barrier against invading pathogens (Bakken, 1981; Seykora and McDaniel, 1985; Constable *et al*., 2017). Generally, the occurrence of mastitis is higher in cylindrical-shaped teats than the funnel-shaped teats (Hickmann, 1964; Rathore, 1976; Bakken, 1981). This might be due to the most frequent teat orifice prolapse in cylindrical-shaped teats (Rathore, 1977; Bakken, 1981). According to Constable *et al.* (2017), the efficiency of the teat defense mechanism depends on the integrity of teat tissue; and its impairment leads to an increase in the risk of intramammary infection.

The multicollinearity test showed that the age of the cow and parity, udder position and age, and udder position and parity were found collinear to each other. This might indicate that these variables are confounding one another. So the multivariable logistic regression analysis revealed that the stage of lactation, teat end morphology, milk yield per day, and udder position were significantly ($p< 0.05$) influencing the occurrence of mastitis. These independent variables are the best predictor of mastitis in lactating cows in the Silte zone, Ethiopia.

Among the isolated bacteria, especially *Staphylococcus* spp. and *Streptococcus* spp. are commonly reported from various parts of the country (Abera *et al.*, 2012; Abunna *et al.*, 2013; Zenebe *et al.*, 2014; Belay *et al.*, 2022; Demil *et al.*, 2022; Girma and Tamir, 2022; Tora *et al.*, 2022).

Conclusions

This study revealed that more than half (50%) of examined lactating cows were affected by mastitis, clinical and subclinical mastitis. Subclinical mastitis was the dominant type of mastitis in the study area. Relatively, more proportion of the hind quarter of the udder is affected by mastitis. The prevalence of mastitis increased with the increasing of parity number and age of cows. Mastitis is significantly higher during the early period of lactation, in cows with round and flat teat ends, and pendulous udder, and in cows producing more than ten liters of milk per day. *Staphylococcus aureus* (29.5%) was the major bacterial agent isolated from affected cows.

Based on this study finding the following recommendations are forwarded: Proper attention should be given to mammary gland health by the owners to reduce the burden of mastitis. Strengthening animal health extension work to aware dairy owners about regular testing for early detection of mastitis, and improving the management of dairy cows will enable to reduce of the problem of mastitis. Dry cow therapy should be advocated and introduced into the areas, which will reduce the prevalence of mastitis during the lactation period. Further study for antimicrobial susceptibility of the common isolates is very important that is for designing an appropriate drug selection and treatment regime.

Limitations of the study

Antimicrobial susceptibility of isolates is not done that is due to budget constraints.

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Ethical consideration

Informed consent was obtained from the animals' owners

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