Isolation, Identification and Antimicrobial Resistance Profile of staphylococcus aureus and Occurrence of Methicillin Resistant S. aureus Isolated from Mastitic Lactating Cows in and Around Assosa Town, Benishangul Gumuz Region, Ethiopia

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Abstract
A cross - sectional study was conducted from November 2016 to May 2017 in Dairy cattle in and around Asossa town in order to estimate the prevalence of mastitis, to identify *S. aurues* from mastitic lactating cows, to evaluate its antimicrobial resistance pattern and to identify risk factors associated with mastitis. A total of 384 Dairy cows milk samples were collected using purposive sampling techniques. The overall prevalence of mastitis at cow level was 39.32 % with 11.45 % and 27.86 % of clinical and subclinical mastitis, respectively. In this study, the subclinical mastitis was significantly higher than clinical mastitis. For all except Age and parity, the multivariable logistic regression analysis for intrinsic and extrinsic risk factors showed significant difference in the prevalence of mastitis in the study area (P<0.05). From 151 mastitis infected lactating cows, 436 milk samples were cultured and 22.14% *S.aureus* were isolated. Presumptively identified *S. aureus* isolates were subjected to antimicrobial susceptibility test and 63(74.11%) MRSA have been identified from a total of 85 *S. aureus* using cefoxitin through disk diffusion method. The present result showed a significant association of resistance pattern with *S.aureus* isolates, particularly to penicillin G (95.55%), Cefoxitin (77.19%), Tetracycline (63.41%), Streptomycin (60.78%), Gentamycin (59.37%), Vancomycin (56.75%), Clindamycine (54.35%) and Bacitracin (53.65%). In this study, 77.19 % *S.aureu*s isolates were found resistant against Cefoxitin. There were also observed multidrug resistance, mainly to Penicillin G, Streptomycin and Tetracycline. The present study revealed higher prevalence of mastitis and occurrence of multidrug resistance *S.aureus* specifically which belongs to the MRSA which are dependent on multiple associated risk factors. Hence, regular resistance follow-up, using antimicrobials sensitivity tests helps to select effective antimicrobials and to reduce the problems of drug resistance developments towards commonly used antimicrobials.
Keywords: Asossa, Antimicrobial susceptibility, Dairy cattle, milk, mastitis, Methicillin resistance, Staphylococcus aureus

Introduction
Ethiopia has the largest cattle population in Africa with an estimated population of 52.13 million [9] and contributes 40 % to the annual agricultural output, and 15% total gross domestic product. Cattle produce a total of 1.5 million tonnes of milk and 0.331 million tonnes of meat annually. Cows represent the biggest portion of cattle population of the country, around 42% of the total cattle heads are milking cows [9]. However, milk production often does not satisfy the country's requirements due to a multitude of factors. Mastitis is among the various factors contributing to reduced milk production [7]. Bovine mastitis is an infectious inflammation or irritation of the mammary glands that interferes with the normal flow and quality of milk. Among mastitis causing pathogens, the *S.aureus* bacterium is a major pathogen of intramammary infections in dairy cattle It is an important opportunistic pathogen both in humans and in dairy cattle [14,37]

S. aureus is present in a variety of locations in the dairy farms, in many occasions it was isolated from swabs taken from the cows head, skin swabs, legs and nasal mucosa and also on the milkers' hands. However an infected udder quarter remains the main reservoir of the bacteria, which transmitted mostly during the milking time [17].

The determination of antimicrobial susceptibility of clinical isolates is required not only for therapy but also for monitoring the spread of resistant strains throughout the populations. ß-lactam antibiotics are the most frequently used in intramammary infusion therapy. Bacterial resistance mechanisms to this class of antibiotics include production of ß-lactamase and low-affinity penicillin-binding protein 2a (PBP 2a) determined by the presence of the chromosomal gene mecA [29].

Recently, increasing evidences point to domestic animals including food animals as reservoirs and shedders of MRSA, and transmission between host species also may be possible. Over the past decade, a growing number of MRSA isolates have been reported in companion and food animals and in their human associates, including pet owners, farmers, and veterinary personnel [25].

MRSA strains have been observed to be multi-drug resistant, such as aminoglycosides, macrolides, lincosamides, streptogramins, tetracyclines, etc., which are often used in the treatment of mastitis [41]. The

usage of antibiotics correlates with the emergence and maintenance of antibiotic resistant traits within pathogenic strains [34].

Few studies have been carried out in some areas of Ethiopia to assess the status of *Staphylococcal* mastitis [1,2,3,11]. However, there was any study done to assess the status of *S. aureus* and/or MRSA in and around Asossa districts, Benishangul Gumuz regional state, Western Ethiopia. Therefore, the objectives of the present study were to determine the prevalence of bovine mastitis, to isolate and identify *S. aureus* from mastitic lactating cows, to assess the risk factors associated with *Staphylococcus* infections and to determine antimicrobial resistance pattern of *S. aureus* species

Materials and methods
Study Area
The study was conducted in and around Asossa Town. Asossa is the capital city of the Benishangul-Gumuz Regional State and composed of 74 administrative peasant associations, which is located at 8°30'and 40°27' N latitude and 34°21' and 39°1' E longitude 687 kms Northwest of Addis Ababa [9]. The altitude of Asossa ranges from 580 to over 1560 meter above sea level. The area is characterized by low land plane agro- ecology which has 'kola' micro climate with land covering 2317 km2 area, according to National Meteorological Service Agency [32] with average annual rainfall of 850-1316 mm with uni-modal type of rainfall that occurs between April and October. Its mean annual temperature ranges between 16.75°C and 30°C. Asossa zone has 35.6% of the livestock population of the region constituting 61, 234 cattle, 191, 83 goats, 19,729 sheep, 25,137 donkeys, 439,969 poultry and 73,495 beehives [9]

Study Design
A cross - sectional type of study design was used from November 2016 to May 2017.

Study animals
Lactating cows of both breeds namely cross breed (Holstein-Friesian - zebu crosses) and local zebu breed were included during the study period.
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Sample size determination and sampling strategy
The study sites were selected purposively based on availability of dairy cows, accessibility, permission of owners and disease presence or absence. Purposive sampling technique was applied small-scale dairy farms dairy farms available in the study area. Henc 384 lactating cows of which 268 indigenous zebu and 116 Holstein - zebu cross cows were selected from 10 different peasant association owing small-scale dairy farmstead and were.

Clinical Inspection of the Udder
Udders of the cows were examined by visual inspection and palpation for the presence of any abnormalities. In addition, milk from each quarter was withdrawn and checked for any change in color and consistency [35]. Clinical cases were recorded at the time of milk sampling. Theses Clinical mastitis cases were diagnosed on the basis of manifestation of visible signs like inflammation of udder characterized by warm and swollen with painful upon palpation and/or gross changes in milk was well considered otherwise chronic mastitis when misshaped, atrophied, hard and fibrotic quarters were examined [36].

California Mastitis Test (CMT)
CMT was conducted to diagnose the presence of subclinical mastitis and it was carried out according to standard procedures. A squirt of milk from each quarter of the udder was placed in each of four shallow cups in the CMT paddle and an equal amount of the reagent was added. A gentle circular motion was applied in a horizontal plane. Positive samples showed gel formation within a few seconds. The result was scored based on the gel formation and categorized as negative if there was no gel formation, or positive if there was gel formation ranging from +1 to +3. If at least one quarter was positive by the CMT then the cow was considered as positive [35].

Sample collection, transportation and handling
For lactating cows, milk samples were collected by purposive sampling method. Aseptic procedure was followed when collecting milk samples in order to prevent contamination with micro organisms present on the skin udder and teats, on the hands of samplers and on the barn environment. Teat ends were cleaned and disinfected with ethanol (70%) before sampling. Strict foremilk (first jets) were discharged to reduce the number of contamination of teat canal [35]. Sterile universal bottle with tight fitting cups were used. The universal bottle was labeled with permanent marker before sampling. To reduce contamination of teat ends during sample

collection, the near teats were sampled first and then followed by the far ones [35].

Milk samples were collected from each of clinically and sub clinically mastitic non-blind quarters of the selected lactating cows for bacterial isolation according to the [31]. After milking out and discarding the first two drops, about 2ml of milk were tested on CMT paddle from each quarter and about 20 ml of milk were aseptically collected from each mastitis positive quarter using sterile universal bottle. Finally, the milk samples were properly transported immediately in an ice box to Regional Veterinary Laboratory of Benishangul Gumuz, Asossa for microbiological examination.

Culturing and Biochemical tests

Isolation and identification of *S. aureus* was conducted by direct streaking of loopful of milk onto 7% sheep blood agar (Oxoid, UK) and incubating aerobically at 37^0C for 24–48 hours. The bacteriological media used was prepared according to the manufacturer's recommendations [35]. The plates were examined for the presence of *Staphylococcus* colonies. Presumed staphylococcal colonies were then subcultured on nutrient agar plates (NAP) and incubated at 37^0C for 24-48 hours to get a pure culture (clone of cells derived from a single cell). After growth of presumptive colonies were identified by using conventional bacteriological techniques on the basis of colony characteristics, pigment production and hemolysis. The final identification of the Staphylococci organisms and *S. aureus* species assignment were done based on Gram staining, catalase test, O-F glucose test, oxidase test, sugar fermentation and coagulase test. Pure cultures of a single colony type from the NAP were inoculated into nutrient slants and incubated at 37^0C for 24-48 hours under aerobic culture conditions. The pure isolates in the nutrient slant were preserved and maintained at $+4^0$C for further need [16,35,38].

In vitro antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for *S. aureus* isolates (N=63) by disc diffusion method, according to the criteria of the Clinical and Laboratory Standards Institute [10]. It was stated in the absence of Methicillin the best alternative is to use Cefoxitin for MRSA identification. The following antibiotics were used for testing: Cefoxitin (Fax/30μg), Vancomycin (VA/30μg), Penicillin G (10U), Tetracycline (TE/30μg), Streptomycin (S/10μg), Chloramphenicol (C/30μg), and Sulphamethoxazole - trimethoprim (SXT/25μg), Cloxacillin (OB/5μg), Clindamycin (DA/10μg), Kanamycin (K/30μg), Gentamycin (CN/10μg) and Bacitracin (B/10μg) Oxoid Company (Hampshire, England).

Colonies isolated from pure culture were transferred into a test tube of 5ml nutrient broth and colony suspension were made and incubated at 37^oc for 8 hours. The turbidity of the direct colony suspension of the isolates were adjusted comparing with that of turbidity equivalent to a 0.5 McFarland standards to antimicrobial agents were determined for isolated strains by the disk diffusion method. Muller-Hinton Agar plates were prepared and a sterile cotton swab was dipped into the suspension and swabbed onto the surfaces of Muller-Hinton Agar plate. Then, the antimicrobial discs were placed onto the agar plate using sterile forceps and pressed gently to ensure the complete contact with the agar surface. The plates were read after 24 hours of incubation at $35\ ^0$C under aerobic condition.

According to the manufacturer's instructions, the diameters of the zone of inhibition around the antibiotic disks were measured to the nearest millimeter using caliper, the isolates were classified in accordance with the guideline [10,35] as susceptible, intermediate or resistance for each antimicrobial drug tested. Intermediate results were considered as resistant. Moreover, multi drug resistant phenotypes were recorded for isolates showing resistance to two or more antimicrobial drugs.

Data Management and Statistical Analysis

Microsoft excel was used for data management, computation of descriptive statistics and drawing graphs. Data was coded and entered to MS Excel spreadsheet and checked for accuracy. After validation, it was transferred and processed using computer software SPSS version 20.0 for analysis. The Pearson's Chi- square (χ2) were used to measure the association between the different risk factors and occurrence of *S. aureus* and/or MRSA in dairy cattle. Odds ratio and 95% CI computed and the 95% confidence level was used. Furthermore, multivariate logistic regression was used to see the association of the potential risk factors with occurrence mastitis. In all analysis, associations were considered to be significant when p<0.05.

Results

Prevalence of mastitic Dairy cows

In this cross- sectional study, out of the total lactating cows examined, 151(39.32 %) mastitis prevalence was found to be affected with clinical and subclinical mastitis infection. During laboratory examination, 85 (22.14%) of the *S.aureus species was isolated* and identified. The proportional prevalence of *S. aureus* species was 85/151(56.29%) and it was found to be statistically significant (P<0.05) (table 1)

Prevalence of mastitis at cow level

The overall prevalence of mastitis at cow level as determined by CMT and clinical examination was 151 (39.32 %) from a total population of 384 cows; 44 (11.45%) were clinical where as 107 (27.86%) were subclinical mastitis and 233 (60.67%) was healthy cows. The relative prevalence of each mastitis type in cows was 29.2% and 70.86% clinical and sub clinical mastitis respectively (figure 1)

Prevalence of MRSA at Quarter level

MRSA was identified using Cefoxitin disk diffusion method (CLSI, 2012). The overall prevalence of Methicillin resistance *S. aureus* at quarter level was 245 (15.95%). The proportional prevalence of MRSA in quarter was 245/436(56.2%). From the four quarters the right hind (31.43 %) shows more prevalent and followed by right front (26.12%), left hind (22.86%), and Left front (19.59 %), respectively.

Intrinsic Risk Factors Associated with mastitis Prevalence

Prevalence of mastitis related to the specific risk factors were determined as the proportion of affected cows out of the total examined. The questionnaire survey and observation data result shows breed, lactation stage, and pregnancy status are amongst the potential risk factors, which are associated with mastitis disease in dairy cows farmstead. Accordingly, mastitis prevalence showed significant variation among different breed groups (p = 0.002), lactation stage (p=0.027), and pregnancy status (p=0.007). However, age and parity have no significant difference with mastitis (p>0.05) (table 2)

Extrinsic Risk Factors Associated with mastitis Prevalence

Management factors like milking hygiene, floor type and antibiotic used or previous mastitis and treatment history were evaluated as risk factors that influence the prevalence of bovine mastitis. Identified extrinsic risk factors have significance on the prevalence of bovine mastitis in the study area (P< 0.05) (table 3)

Isolation of *S. aureus* from mastitic dairy cows

From 151 mastitis cases, infected Dairy cows (clinical 44 cows, subclinical 107 cows of 436 milk samples) were cultured and 85 *S. aureus* were isolated. The proportional isolates of *Staphylococcus aureus* in clinical and subclinical mastitis was 85/151 (56.29 %). *S. aureus* was isolated at a rate of 24/151 (15.89%) and 61/151(40.39%) from clinical and subclinical mastitis infections, respectively (Figure 2)

In vitro Antimicrobial Susceptibility Test result

From a total of 85 isolates of *S. aureus* obtained from the study antimicrobial susceptibility tests were performed on 63 isolates. Due to the relatively small size, no separate analysis was undertaken for clinical and sub clinical isolates of *S.aureus* and were tested for antimicrobial sensitivity for 12 different types of Antimicrobials. The present study has demonstrated the existence of the levels of resistance of *S.aureus* to commonly used antimicrobial agents in the study area. 77.19 % of the *S. aureus* was found to be resistance to Cefoxitin, which shows the prevalence of MRSA. The resistance profile of Penicillin G, Tetracycline, Streptomycin, Gentamycin, Vancomycin, Clindamycin and Bacitracin were 95.55%, 63.4%, 60.78%, 59.37%, 56.75%, 54.35 and 53.65 %, respectively. In this study, *S. aureus* were found to be highly susceptible to Chloramphenicol (77.27%), Cloxacillin (70.58%), Trimethoprim-sulfamethoxazole (65.0%) and followed by Kanamycin (58.62%). However, these isolates were highly resistant to penicillin G (95.55%) and Cefoxitin (77.19%) followed by Tetracyline (63.41%) (Table 4)

Association of Cefoxitin resistance with previous treatment

From a total of 151 (39.32%) CMT positive dairy cows, 90 /151 (59.60%) of cows were found to be previously treated with antibiotics and also from a total of 233/384 (60.67%) cows which shows susceptibility to Cefoxitin, 229/233 (98.28%) cows were cefoxitin susceptible without previous treatment. The *invitro* Disc sensitivity test result shows 44 (77.2%) isolate were found to be resistant to Cefoxitin. Therefore, MRSA was found to be associated with previous treatment history of the animal with Cefoxitin resistance (table 5).

Discussion

In the present study, the overall prevalence of mastitic dairy cows was 39.32 % in cows. This result was in line with a prevalence of 40% reported by [22] in Southern Ethiopia (40%) in cows. This report is relatively similar with the assertion by [36] that in most countries and irrespective of the cause, the prevalence of mastitis is about 50% in cows and 25% in quarters. Besides, this result was in line with the findings of [8] at Bahir Dar and [30] around Wolaita Sodo, 28.8%, 29.5% in cows respectively. However, the current prevalence is lower when compared with the previous findings as 56% in and around Kombolcha town [40]. This variability in prevalence

of mastitis between different reports could be attributed to differences in farms management practice or to differences in study methods agro-climatic condition. As mastitis is a complex disease involving interactions of various factors such as managemental and husbandry, environmental conditions, animal risk factors, and causative agents, its prevalence will vary [36].

The occurrence of clinical mastitis in the present study was 11.45% (44/384) and that of subclinical mastitis was 28.0 % (107/384) at cow level. This also provides further support of other studies in different region of the country which have concluded that subclinical mastitis is more prevalent than clinical mastitis. [2,7,12,26,40] who have reported as (46% , (36.7% , (23.0% v , (48.6% and (73.3% subclinical and clinical mastitis, respectively.

The prevalence of clinical mastitis in cows is in line with reports made by [40] in and around kombolcha, [22] in Southern and [20] in central Ethiopia with a rate of 10%, 10% and 16.11% respectively; but comparably lower findings of clinical mastitis at cow level is reported as 6.48%, and 4.8% in Bahir Dar, and in and around Batu town respectively [8] and 3.9% in Adama [2]

This variation in prevalence between subclinical and clinical mastitis may be due to the fact that, the defense mechanism of the udder reduces the severity of the disease [14].

In this study, the prevalence of subclinical mastitis in cross and local breeds at cow level were 50.6% and 34.4% respectively whereas prevalence of clinical mastitis in cross and local breeds were 51.5% and 21.5% respectively. this report is lower as compared to the findings of [23] in smallholder dairy farms in Tanzania who reported prevalence of 90.3% and in cross breeds of cows

The current study as well as in other similar studies, overwhelming cases of mastitis were subclinical as compared to clinical mastitis in both breeds [22,42].

In the present study, the prevalence of *S. aureus* in subclinical mastitis was 61/151 (40.39%) significantly higher than clinical mastitis 24/151 (15.89%). This is due to *S. aureus* is adapted to survive in the udder and usually establishes chronic subclinical infection of long duration from which it is shaded through milk serving as sources of infection for other healthy cows and transmitted during the milking process [36].

With regard to the bacteriological analysis of milk sample, the relative isolates of *S.aureus* were 85/151(56.3%). This finding is inconsistent with the earlier findings of in Holleta agricultural research centre (43.3%) by [12] in Hawassa area (48.75%) by [11] and in Holeta town (47.1%) by [26]. Similarly, this result was inline with the previous findings of [22,5,42] who have reported as 40.3%, 39.1% and 39.2% and *S. aureus* isolates at Assela, Addis Ababa and Southern Ethiopia, respectively. It was also closely comparable with findings of [24,33] who reported 41.1% and 43.3% in dairy cows, respectively.

An increased occurrence of MRSA was found to be associated with previous treatment history of the animal. All of the isolated MRSA were from adult and old age category and no susceptibility is recorded in old age and also 59.6% of dairy cows were previously treated while 60.7 % of cows were cefoxitin susceptible without previous treatment. These may be due to the fact that prolonged time of survival under low managemetal condition for dairy cattle leads to a possible chance of exposure to mastitis infection, so possibility of repeated antibiotic treatment will be relatively higher in aged animals [37, 36].

In this study, floor system had a significant influence on the occurrence of mastitis. In agreement with [4,24]. The findings of a high prevalence of mastitis in farms with muddy (soil) floors (49.12%) when compared with concrete floor types (25%) shows the occurrence of mastitis is significantly associated with the housing (bedding) type or condition of the farm. This is due to association with poor sanitation and cows which were maintained in dirty and muddy common barns with bedding materials that favor the proliferation and transmission of mastitis pathogens. The main sources of infection are udder of infected cows transferred via milker's hand, towels and environment [36]. Occurrence of mastitis was significantly associated with milking hygienic practice. Cows at farms with poor milking hygiene standard are severely affected (53.69%) than those with good milking hygiene practices (23.20 %) [15,24, 30]. This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis [36].

In this study, the *in vitro* disc sensitivity test showed that only two drugs have shown less degree of resistance, 0 to 25% of the total isolates tested. These drugs were Chloramphenicol (6.82%) and Cloxacillin (17.64%) and followd by Sulphamethoxazole -trimethoprim (27.5%). Similar results with the finding of [1,2,27] who reported less résistance of Chloramphenicol and Sulphamethoxazole- trimethoprim. The reason why these antimicrobials were less resistant might be that they are not used in the study area in veterinary clinics or services and even not frequently used (infrequent use of therapeutics) perhaps in human medicine. The present study showed that the resistance of *S. aureus* to Penicillin G (95.6%), Cefoxitin (77.2%), Tetracycline (63.4%), Cloxacillin (17.6%), Streptomycin (60.8%), Sulphamethoxazole -trimethoprim (27.5%), Chloramphenicol (6.8%), Kanamycin (31.0%), Vancomycin (56.8%), Bacitracin (53.7%), Gentamycin (59.4%) and Clindamycin (54.4%) observed in milk samples. This results were in consistent with reports from earlier studies in the other countries [13,39] suggesting a possible development of resistance from prolonged and indiscriminate usage of

some antimicrobials. The resistance of *S. aureus* isolates to beta-lactam antibiotic was evident. High percentage of *S. aureus* was resistant to the most frequently used drugs. All cefoxitin resistant *S. aureus* were also resistant to penicillin G. Out of the (77.2%) cefoxitin resistant *S. aureus* isolates, (95.6%) of were also resistant to Penicillin G. This is an indicator of MRSA [6,18,28]. This is due to the fact that resistance of *S. aureus* to these drugs may be attributed to the production of β-lactamase, an enzyme that inactivates penicillin and closely related antimicrobials [19, 21].

In coclusion, it was found that the majority of the tested isolates were resistant to the multiple antimicrobial agents. Hence, regular antimicrobial sensitivity test to select effective and alteration of antibiotics must be carried out, and the impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

Acknowledgents

Competing interests

There is no any conflict of interests among the authors

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Table 1: Prevalence of mastitis at breed level in cross breed and local zebu of lactating cows

Breed	No of animal examined	No of positive (%)	X^2	p-value	95% CI
cross breed	116	59 (50.86%)	9.27	0.002	0.324-0.786
local zebu	268	92 (34.32%)			
Total	384	151 (39.32%)			

Table 2: Result of multivariate logistic regression of attribute risk factors with mastitis

Factor	Categories	Total no examined	No (%) positives	OR	X^2	p-value	95% CI
Age (years)	≥3- 5 (y-ad)	136	52 (38.23%)	1.146	1.402	0.49	0.789-1.665
	>6 - ≥9 (adult)	231	90 (38.96%)				
	> 9 (old)	17	9 (52.94%)				
Breed	Cross	116	59(50.86%)	0.505	9.27	0.002	0.324-0.786
	Zebu	268	92(34.32%)				
Parity	1-2	210	80(38.09%)	1.164	2.06	0.357	0.87-1.54
	3-4	120	45(37.5%)				
	≥5	54	26(48.14%)				
Lactation Stage (m)	Early (≤3)	127	61(48.03%)	0.914	9.19	0.027	0.740-1.129
	Mid (4-6)	135	41(30.37%)				
	Late (7-9)	82	31(37.80%)				
	Dry (>9)	40	18(45%)				
Pregnancy Status	Pregnant	103	29(28.15%)	0.51	7.35	0.007	0.312-0.834
	Non- Pregnant	281	122(43.41%)				

Key: OR**:** odd ratio, CI**:** confidence interval

Table 3: Result of extrinsic risk factors with the occurrence of mastitis.

Factor	Categories	Total no examined	No (%) positives	OR	X^2	p-value	95% CI
Previous mastitis History		141	136(96.45%)				146.98-1162.89
	Infected			413.44	304.80	0.000	
	Non- infected	243	15(6.17%)				
Floor type	Concrete	156	39(25.0%)	0.345	22.58	0.000	0.221-0.539
	Muddy (soil)	228	112(49.12%)				
Milking hygiene	Good	181	42(23.20%)	0.260	37.28	0.000	0.167-0.405
	Poor	203	109(53.69%)				
Prevoius mastitis Rx history	Yes	94	90(95.74%)	84.46	166.06	0.000	29.83-239.11
	No	290	61(21.03%)				

Key: OR**:** odd ratio, CI**:** confidence interval

Table 4: Resistance of *S. aureus* isolates to different antimicrobials (n = 63).

Antimicrobial agents	Resistance	Intermediate	Susceptible
	No (%)	No (%)	No (%)
Cefoxitin	44 (77.19)	0	19 (30.15)
TTC	26 (63.41)	3 (7.32)	12 (29.26)
Cloxacillin	6 (17.64)	4 (11.76)	24 (70.58)
Clindamycin	25 (54.35)	8 (17.39)	13 (28.26)
Gentamycin	19 (59.37)	4 (12.5)	9 (28.12)
Streptomycin	31 (60.78)	8 (15.68)	12 (23.52)
Penicillin G	43 (95.55)	2 (4.44)	0
Chloramphenicol	3 (6.82)	7 (15.90)	34 (77.27)
Kanamycin	9 (31.03)	3 (10.34)	17 (58.62)
SXT	11 (27.5)	3 (7.5)	26 (65.0)
Vancomycin	21 (56.75)	4 (10.81)	12 (32.43)
Bacitracin	22 (53.65)	6 (14.63)	13 (31.70)
Mean	260 (21.66)	52 (4.33)	191(15.91)

Key: SXT- Trimethoprim-sulfamethoxazole, S- Susceptible, I- Intermediate, R- Resistant

**Table 5**: Drug resistance pattern of S. aureus and age of cows

Age of cow	*S. aureus* isolated	Resistance pattern			p-value	Df	X^2
		One drug	Two drug	Multi drug			
Young	13	9 (69.23%)	4 (30.76%)	0 (0%)	0.0001	2	0.92
Adult	41	4 (9.75%)	8 (19.51%)	29 (70.73%)			
Old	9	0 (0%)	0 (0%)	9 (100%)			
Total	63	13	12	38			

Key: X^2 = 0.92, Df = 2, P-value = 0.0001, statistically significant (P<0.05).Multidrug resistance prevalence were =38*100/63 = 60.32%

Table 6: Association of Cefoxitin resistance pattern with previous treatment

Cefoxitin resistance	Previous mastitis treatment		
	Yes	No	Total
Positive/Resistance pattern per cow	90	61	151
Negative/susceptible pattern per cow	4	229	233
Total	94	290	384

Key: X^2 = 166.06, df=1, P-value = 0.000, OR= 84.467, 95%CI=29.83-239.11, statistically significant (p<0.000).
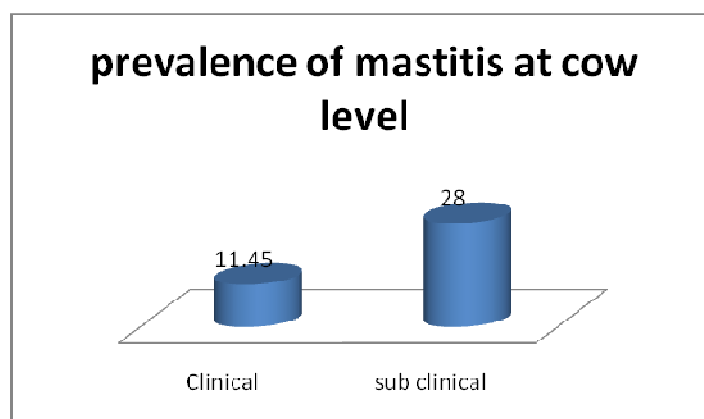
Figure 1*:* Prevalence of different types of mastitis (n=151)

Figure 2: *S. aureus* Gron on Mannitol salt agar medium