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Evaluation of autovaccine against mastitis causing *Staphylococcus aureus* in and around Tirupati, Chittoor district, Andhra Pradesh

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Abstract
The aim of the study was to prepare and evaluate the efficacy of auto vaccine prepared against *Staphylococcol* mastitis causing of *S. aureus*. Using local isolates of the selected areas in and around Tirupati, Chittoor District within smaller groups of dairy cows. Whole cell *S. aureus* auto vaccine was prepared for selected therapeutic areas in and around Tirupati, Chittoor District using local isolates. The evaluation of auto vaccine was carried out against mastitis of *S. aureus* origin in eliminating *S. aureus* from milk of 27 cows with subclinical mastitis. The antibiogram results of *S. aureus* isolates in Chittoor district shown higher sensitivity to beta-lactam antibiotics like Amoxycillin (60.37%) followed by Ampicillin (56.60%) whereas in selected therapeutic areas higher sensitivity to *S. aureus* isolates was observed to quinolones like Enrofloxacin (66.66%) and Ciprofloxacin (59.25%). Enrofloxacin was selected as antibiotics of choice based on antibiotic susceptibility test of *S. aureus* isolates from therapeutic areas. The therapeutic trials were conducted in three divided groups of 9 cows each, group-I was given with antibiotic enrofloxacin, group-II was given with *S. aureus* auto vaccine and group-III was given with both enrofloxacin and *S. aureus* auto vaccine.
The antibiotic was administered parenterally, whereas auto vaccine was given subcutaneously nearer to the upper udder lymphnode region. After 35 days of the treatment *S. aureus* was not detected in the milk of 33.3% of the cows treated with enrofloxacin, 66.7% of the cows treated with *S. aureus* auto vaccine, and 100% of the cows that underwent combined therapy of Enrofloxacin and auto vaccine. The study results shown that combined therapy of antibiotic and auto vaccine was more effective in eliminating *S. aureus* from milk of *S. aureus* infected cows compared to either of them alone.

Keywords: Autovaccine, mastitis causing, *Staphylococcus aureus*

Introduction
Mastitis is the most frequent reason for antimicrobial drug use in dairy herds and associated with antimicrobial resistance due to improper usage of antimicrobial agents. Therefore, it is important to monitor antimicrobial susceptibility of mastitis pathogens (Li *et al.*, 2007) [12]. Several therapeutic measures have been exercised for the prophylaxis and treatment of the mastitis. The wide spread use of antibiotics for treatment of mastitis leads to the development of bacterial resistance and affected animal leads to antibiotic residues in milk affecting human health. Such concerns have prompted the World Health Organization (WHO, 2015) [22] to issue recommendations on global programs to reduce the use of antibiotics and it is plausible that antibiotic therapies to animals will be restricted in the future especially as prophylactic agent (Twomey *et al*., 2000) [21]. Such a limitation would require alternative therapies to treat and control mastitis.
A range of antibiotics are used in mastitis treatment, but due to increasing antibiotic resistance in mastitis causing bacteria the therapy is not always effective. Therefore, attempts have been made to use auto vaccines as an alternative to antibiotics (Nawrotek *et al*., 2012) [15]. Therefore, the attractive alternative includes the administration of autogenous vaccines to the mastitis cows and use of herbal drugs for the treatment of mastitis. Auto vaccines are known to be useful in treating many different infections (Nolte *et al*., 2001) [14].
Vaccines against *Staphylococci* have been studied and suggested as an important tool in the management of *Staphylococcal* infections in dairy cows (Daum and Spellberg, 2012) [5]. Experimental challenge studies with *Staph. aureus* have shown an effect of vaccination on the amount of bacterial shedding after challenge (Pérez- Casal *et al*., 2006) [6]; however, such experimental studies were not able to demonstrate a reduction in transmission of infection. Several study designs to estimate vaccine efficacy of contagious infections have been proposed

(Halloran *et al*., 1998) [7]. Since, *Staphylococcus aureus* is considered as a major etiological factor in mammary gland inflammation (Kauf *et al.,* 2007) [10]. To date, several different vaccines have been used for prophylaxis of mastitis (Perez-Casal *et al*., 2006) [6]. However, the commercially available anti-*S. aureus* vaccines are incapable of providing complete protection against mastitis (Middleton *et al*., 2009) [13].
To overcome this, an attempt was made to prepare an auto vaccine with local isolates to eliminate intramammary infection of udder caused by *S. aureus*.

Materials and Methods

Collection of milk samples

Milk samples were collected randomly from organized, unorganized sectors in milch cattle from all the Mandals of Chittoor District, Andhra Pradesh State during the period from November 2017 to July 2018 and subjected for screening against clinical and subclinical mastitis with CMT. Later isolation was carried out according to the method described by Cruckshank *et al*, 1975) [4].

In-vitro antibiogram pattern of *Staphylococcus aureus* isolates from clinical and sub clinical mastitis cases of cows in therapeutic areas.

The antibiotic sensitivity of *S. aureus* isolates were tested as per the method of Bauer *et al*. (1966).The antibiotic discs used for the study were procured from Hi media laboratories Ltd., Mumbai, India. (Table-1).

In-vitro antibiotic sensitivity test

1ml of 24 hrs nutrient broth culture was uniformly spread over Muller Hinton agar (MHA) with sterile cotton swab. Antibiotic discs were placed on inoculated agar surface at about 2cm apart. The plates were incubated at 37 ^{0}C overnight and examined for zone of inhibition. Diameter of the zones of inhibition measured for each antibiotic. The measurements were compared with zone size interpretative chart furnished by the manufacturer and the zones were graded as sensitive (S) or resistant (R).

Evaluation of *S. aureus* autovaccine against subclinical mastitis

Trial design

Trials were designed at farm level as well as field level for the evaluation of *S. aureus* specific autovaccine prepared from local isolates.

Farm level trial

Nine (9) dairy cows from Livestock Farm Complex (LFC), College Of Veterinary Science (CVSc), Sri Venkateswara Veterinary University (SVVU), Tirupati which positive for subclinical mastitis on CMT were selected for on farm trials.

Field level trial

Nine (9) dairy cows from Sorakayalapalem Village of Ramachandrapuram Mandal and Nine (9) dairy cows from Perur mandal of Tirupati rural which positive for subclinical mastitis on CMT were selected for therapeutic field trials.

Preparation of *S. aureus* autovaccine

S. aureus autovaccine was prepared according to the method described by Czernomysy-Furowicz *et al*., (2014) [4]. *S. aureus* autovaccine was prepared using local isolates as vaccine candidates against *Staphylococcl* mastitis. The local *S. aureus* isolates selected for preparation of vaccine were grown in 10ml of sterile test tubes with BHI broth. The inoculated stock cultures were incubated at 37 ^{0}C for 24 hrs. The cultures containing 3 × 108 CFU/ml were used as inoculum for further cultivation of organisms in preparation of vaccine.
10ml of inoculum thus prepared containing *S. aureus* was inoculated into Rous flasks containing 100ml of BHI agar medium and incubated at 37 ^{0}C for 24 hrs. Following, incubation the bacterial growth from the Rous flasks was scrapped into a sterile 0.85% saline solution. Then concentration was adjusted to 3 × 108 CFU/ml with McFarland standards (Hi media Laboratories Pvt. Ltd).

Formalin inactivation

The formalin inactivation of *S. aureus* culture was done according to the method described by Czernomysy-Furowicz *et al*., (2014) [4]. The bacterial cultures thus obtained were inactivated by adding 0.5ml of formalin (0.5%) to 100ml bottle containing *S. aureus* suspension. After incubation and centrifugation the formalized *S. aureus* cells were resuspended in 0.85% Normal saline to a concentration of 3 × 108 CFU/ml.

Standardization of *S. aureus* autovaccine

Standardization of the autovaccine was done according to Czernomysy- Furowicz *et al*., (2014) [4].

Sterility testing of *S. aureus* autovaccine

The sterility of *S. aureus* autovaccine was confirmed by seeding 0.1ml of vaccine on to Blood agar, Nutrient agar, Sabouraud`s dextrose agar and Thioglycollate broth in duplicates of which each one kept at room temperature (25 ^{0}C to 35 ^{0}C) and at 37 °C for 14 days and checked for any microbial growth.

Safety testing of *S. aureus* autovaccine

Safety of the vaccine was studied in mice. Double the normal dose of the vaccine (0.4ml) was given subcutaneously to the mice, and observed for any local or systemic reactions and toxicity at 1hr, 2hr and 4hrs after post inoculation and twice daily for 2 weeks.

Evaluation of *S. aureus* autovaccine

Farm level vaccine therapy

A. Livestock Farm Complex (LFC), CVSc, SVVU, Tirupati.
The animals which were positive for subclinical mastitis on CMT were divided into 3 groups of 3 cows each. Each group of animals received a different kind of therapy. Group 1 received Enrofloxacin (FloxidinTM VET – Intervet India Pvt. Ltd, Pune, India), group 2 received the *S. aureus*-specific autovaccine, and group 3 received both Enrofloxacin (FloxidinTM VET – Intervet India Pvt. Ltd, Pune, India) and the *S. aureus*-specific autovaccine (Table-2).

Field level vaccine therapy

Two field trials were conducted for autovaccine therapy (Table-2).

a. Sorakayalapalem village of Ramachandrapuram Mandal of Tirupati division of Chittoor district
b. Perur mandal, Tirupati, Chittoor district

Group 1: The antibiotic was given intramuscularly daily 16ml for 7 days.

Group 2: The autovaccine was administered once (at the time of administration of the first dose of antibiotic) in the udder region, subcutaneously 6ml for the therapy.

Group 3: The antibiotic was given intramuscularly daily 16ml for 7 days and autovaccine was administered once (at the time of administration of the first dose of antibiotic) in the udder region, subcutaneously 6ml for the therapy.

Efficacy of *S. aureus* autovaccine

The effectiveness of treatment of autovaccine was evaluated on the basis of the CFU of *S. aureus* per 1ml of milk of the therapeutic cows according to Czernomysy- Furowicz *et al*., (2014) [4] using pour plate method.

The number of *S. aureus* organism present in milk of suspected mastitis positive cows before and after treatment was analysed using automatic colony counter. Milk samples were collected on 0th day of before therapeutic treatment and after 7th, 21st and 35th day of therapeutic treatment for bacterial count.

The milk samples were collected from four quarters of each cow into one container before treatment (day 0) and the 7, 21 and 35 days after treatment. Samples were collected aseptically according to standards and stored at 4 ^{0}C until process. Initially, decimal dilution with 0.85% NaCl (10-1 to 10-9) was performed. Then 0.1 ml of milk from each dilution was plated onto Mannitol salt agar (Hi media, Mumbai) and incubated for 48hrs at 37 ^{0}C. After 48hrs of incubation, the colonies per ml of milk sample were counted using automatic colony counter (Inter science, Labmate Asia Pvt. Ltd).

Statistical analysis

Data are presented as the number of *S. aureus* infected cows before and after treatment in particular groups. The statistical analysis was conducted using chi-square test according to Preacher, (2018) [17].

Preparation of *S. aureus* autovaccine

S. aureus autovaccine was prepared according to the method described by Czernomysy-Furowicz *et al*., (2014) [4]. *S. aureus* auto vaccine was prepared using local isolates as vaccine candidates against *Staphylococcal* mastitis. The local *S. aureus* isolates selected for preparation of vaccine were grown in 10ml of sterile test tubes with BHI broth. The inoculated stock cultures were incubated at 37 ^{0}C for 24 hrs. The cultures containing 3 × 10^8 CFU/ml was used as inoculum for further cultivation of organisms in preparation of vaccine. 10ml of inoculum thus prepared containing *S. aureus* was inoculated into Rous flasks containing 100ml of BHI agar medium and incubated at 37 ^{0}C for 24 hrs. Following incubation, the bacterial growth from the Rous flasks was scrapped into a sterile 0.85% saline solution. Then concentration was adjusted to 3 × 108 CFU/ml with McFarland standards (Hi media Laboratories Pvt. Ltd, Mumbai).

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Standardization of *S. aureus* auto vaccine

Standardization of the auto vaccine was done according to the method of Czernomysy- Furowicz *et al*., (2014) [4].

Sterility testing of *S. aureus* auto vaccine

The sterility of *S. aureus* auto vaccine was confirmed by seeding 0.1ml of vaccine on to Blood agar, Nutrient agar, Sabouraud`s dextrose agar and Thioglycollate broth in duplicates of which each one kept at room temperature (25 ^{0}C to 35 ^{0}C) and at 37 °C for 14 days and checked for any microbial and fungal growth.

Safety testing of *S. aureus* autovaccine

Safety of the vaccine was studied in mice. Double the normal dose of the vaccine (0.4ml) was given subcutaneously to the mice, and observed for any local or systemic reactions and toxicity at 1hr, 2hr and 4hrs after post inoculation and twice daily for 2 weeks.

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Testing of *S. aureus* autovaccine

Safety of the vaccine was studied in mice. Double the normal dose of the vaccine (0.4ml) was given subcutaneously to the mice, and observed for any local or systemic reactions and toxicity at 1hr, 2hr and 4hrs after post inoculation and twice daily for 2 weeks.

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The milk samples were collected from four quarters of each cow into one container before treatment (day 0) and the 7, 21 and 35 days after treatment. Samples were collected aseptically according to standards and stored at 4 ^{0}C until process. Initially, decimal dilution with 0.85% NaCl (10-1 to 10-9) was performed. Then 0.1 ml of milk from each dilution was plated onto Mannitol salt agar (Hi media, Mumbai) and incubated for 48hrs at 37 ^{0}C. After 48hrs of incubation, the colonies per ml of milk sample were counted using automatic colony counter (Inter science, Labmate Asia Pvt. Ltd).

Results

The present study on evaluation of *S. aureus* specific autovaccine against mastitis caused by *Staphylococcus aureus* was evaluated to eliminate staphylococcal mastitis infection in and around Tirupati at farm level as well as in field. The *S. aureus* isolates recovered from the cows with subclinical mastitis cases of therapeutic areas in Chittoor district were phenotypically similar and belongs to one genotype confirmed by 16SrRNA PCR. The *S. aureus* isolates in therapeutic areas which were similar phenotypically and genotypically used in the preparation of autovaccine.

***In-vitro* antibiotic sensitivity test results of recovered *S. aureus* isolates of therapeutic areas (n=27)**

In-vitro antibiotic sensitivity test results of *S. aureus* isolates recovered from therapeutic areas were sensitive to Enrofloxacin 66.66% (18/27) followed by Ciprofloxacin 59.25% (16/27), Amoxicillin 44.44% (12/27), Ampicillin 40.74% (11/27), Pencillin-G 33.33% (9/27), Gentamicin 25.92% (7/27), Tetracycline 18.51% (5/27), Streptomycin 14.81% (4/27), Kanamycin 11.11% (3/27) and Amikacin 3.71% (1/27) and were resistant to Amikacin 96.29% (26/27) followed by Kanamycin 88.89% (24/27), Streptomycin 85.19% (23/27),Tetracycline 81.49% (22/27), Gentamicin 74.08% (20/27), Pencillin-G 66.67% (18/27,) Ampicillin 59.26% (16/27), Amoxicillin 55.56% (15/27), Ciprofloxacin 40.75% (11/27) and Enrofloxacin 33.34% (9/27) respectively (Table-3) (Fig.01 & Fig.2).

Based on antibiotic sensitivity test results was found that all the recovered *S. aureus* isolates of therapeutic areas showed higher sensitivity to Enrofloxacin. Hence, Enrofloxacin containing 100mg of Enrofloxacin was choosen for antibiotic treatment for the cows with subclinical mastitis in the present work. The *S. aureus* auto vaccine safety test in mice indicates the *S. aureus* autovaccine was safe and also sterile on sterility test. (Fig.3).

The major etiological agent was found to be *S. aureus* on isolation and characterization studies. Hence, auto vaccine was prepared using local isolates and enrofloxacin was found to be antibiotic of choice on antibiotic sensitivity test. Hence, 100mg of enrofloxacin was chosen for therapeutic treatment along with auto vaccine. *S. aureus* auto vaccine prepared was found to be safe on safety test in mice.

The results showing the efficacy of auto vaccine against *S. aureus* in the elimination of *S. aureus* from the milk of therapeutic cows in respective groups is presented in the (Table-4) (Fig.4, 5 & 6). The statistical analysis showed statistical significant differences (P = 0.04) between the number of *S. aureus* infected cows in different groups of therapeutic treatment on days 7, 21, and 35 of the trail. It was found that in the group receiving Enrofloxacin, 7 days after the last antibiotic administration *S. aureus* was not found in the milk of 6 cows (66.67%) whereas after 21 and 35 days the number of cows free of *S. aureus* was decreased to 3 (33.33%). Furthermore, the present study showed that, after 7 days of treatment *S. aureus* was not isolated from the milk of 3 cows (33.33%) treated with *S. aureus* auto vaccine. It was also noted that on day 35 of the experiment the number of cows that received the auto vaccine and from which *S. aureus* was not isolated increased to 6 (66.67%). But the most effective therapeutic efficacy was observed in the group receiving antibiotic Enrofloxacin with *S. aureus* specific auto vaccine, in which between days 7 and 35 all the animals were free of *S. aureus*.

Discussion

Mastitis is the predominant intra-mammary infection in dairy cattle in India causing huge economic loss to the dairy industry. Both clinical and subclinical bovine mastitis are responsible for reduction in milk production, deterioration of quality of milk and milk products, increased amount of health care expenditure, financial loss due to culling of sick animals and even mortality of cow. Despite application of various managemental practices and antibiotic therapy, mastitis is still an unresolved battle in dairy industry worldwide including India.

S. aureus is the prominent contagious agent of bovine mastitis. A wide range of antibiotics are used for treatment of mastitis, but due to increasing antibiotic resistance in mastitis causing bacteria the therapy is not always effective. Therefore attempts have been made to use autovaccines as an alternative to antibiotic (Nawtotek *et al*, 2012) [15]. To date, several different vaccines have been used for prophylaxis of mastitis (Perez-Casal *et al*, 2006) [6] However, the commercially available anti S. aureus vaccines are incapable of providing complete protection against mastitis (Middle ton *et al*, 2009).

During the study, antibiotic sensitivity test was conducted on *S. aureus* isolates recovered from in selected therapeutic areas in and around Tirupati. The antibiotic sensitivity test results revealed that the isolates recovered from therapeutic areas shown higher sensitivity to Enrofloxacin 66.66% followed by Ciprofloxacin 59.25%, Amoxicillin 44.44%, Ampicillin 40.74%. Similar sensitivity of *S. aureus* to Enrofloxacin was reported by Baghel *et al*., 2018 [2]; Dar *et al*., 2014 [6]; Bhanot *et al*., 2012 [1]; Kumar and Sharma 2002 [9]; Sahoo *et al*., 2009 [20]; Joshi *et al*., 2006 [8] and Ranjan *et al*., 2010 [18]. It could be due to lesser usage of these antibiotics in majority mandals of Chittoor district in treating the cases of mastitis.

In the present study, *S. aureus* auto vaccines was prepared using local *S. aureus* strains isolated from selected therapeutic areas which were confirmed phenotypically and genotypically showing 95-100% identity with gene bank reference strain.

The present study evaluated the effectiveness of enrofloxacin, (group-I), *S. aureus* auto vaccine (Group-II) and Enrofloxacin + *S. aureus* auto vaccine (group-III) treatment in eliminating mastitis caused by *S. aureus* in dairy cattle of selected areas of Chittoor District in and around Tirupati. It was found that in the group-I receiving enrofloxacin, 7days after the last antibiotic administration S. aureus was not isolated from the milk of 6 cows (66.7%) Antibiotics take effect in the place where the bacterial cells are accessible and don't destroy bacteria in side Phagocytic cells. Enrofloxacin inhibit peptidoglycan biosynthesis, leading to death of unphagocytosed bacteria. However, the Staphylococci located inside the phagocytic cells, could servive due to lack of antibiotic accessibility. After 21 and 35 days of antibiotic treatment, the number of *S. aureus* infection free cows decreased in the antibiotic group from 6 to 3(33.33%).

The present study showed that, after 7 days of treatment, *S. aureus* was not isolated from milk of 3 cows (33.3%) treated with *S. aureus* specific auto vaccine and after 35 days this number increased to 6 (66.7%) animals. The efficacy of the auto vaccine is associated with the presence of antigens, which are the same as the antigens on the surface of *S. aureus* strains that cause the disease. The effectiveness of auto vaccine depends on the place and method of their administration. The efficacy of herd specific auto vaccine in *S. aureus* elimination was 60% reported by (Czernomysy-Furowicz *et al*., 2014) [4] and Mastivac I vaccine with 70% efficacy (Leitner *et al*., 2003) [11] and both the reports were in agreement with the present study with efficacy of 60%.

Auto vaccines were administered through upper udder lymph node region by the earlier workers. Whereas, during the present study the auto vaccine was administered through subcutaneously nearer to the udder lymph node region because to avoid damage of the sensitive udder lymph node and same efficacy of the auto vaccine was reported.

In the present study the most encouraging results were observed in the group-3 received both enrofloxacin and *S. aureus* specific auto vaccine, in which between days 7 & 35, all the animals were free of *S. aureus*. This observation was in accordance with earlier observation made by (Czernomysy-Furowicz *et al*., 2014 and Smith *et al*., 2006) [4, 19] and also reported that this system of therapy resulted in long term protection against *Staphyloccal* mastitis and in the cows selected for the combined therapy no mastitis was observed for at least 2 years. Significant reduction in mastitis cases in combined therapy of antibiotic with auto vaccine is that an auto vaccine and antibiotic can have synergistic action. The reason being that the antibiotic eliminates extra cellular bacteria, whereas the auto vaccine stimulates synthesis of anti *S. aureus* immunoglobulin G2 and enhances phagocytic activity leading to digestion of engulfed (Intracellular) bacteria.

Table 1: Details of Antibiotic discs used in *in-vitro* antibiotic sensitivity test from *S. aureus* isolates

S. No	Name of the antibiotic	Antibiotic symbol	Concentration (in mcg)
1.	Amikacin	AK	30 mcg
2.	Amoxicillin	AMX	30 mcg
3.	Ampicillin	AMP	10 mcg
4.	Kanamycin	K	30 mcg
5.	Ciprofloxacin	CIP	5 mcg
6.	Enrofloxacin	EX	10 mcg
7.	Gentamicin	GEN	10 mcg
8.	Pencillin-G	P	10 units
9.	Tetracycline	TE	10 mcg
10.	Streptomycin	S	25 mcg

Table 2: Details of *S. aureus* specific Autovaccine therapy (Farm/Field)

S. No	Name of the area in Chittoor district	No. of animals (Cows)	Details of Therapeutic groups		
			Antibiotic	Autovaccine	Antibiotic +Autovaccine
1.	LFC, CVSc, SVVU, Tirupati	9	3 no's	3 no's	3 no's
2.	Sorakayalapalem, Ramachandrapuram mandal, Tirupati	9	3 no's	3 no's	3 no's
3.	Perur, Tirupati	9	3 no's	3 no's	3 no's

Table 3: *In-vitro* antibiotic sensitivity test results of recovered *S. aureus* isolates of therapeutic areas (n=27).

S. No	Antibiotic disc	ABST disc code	Conc. of the antibiotic	Sensitive	%	Resistant	%
1.	Amikacin	AK	30mcg	1	3.71	26	96.29
2.	Amoxicillin	AMC	30mcg	12	44.44	15	55.56
3.	Ampicillin	AMP	10mcg	11	40.74	16	59.26
4.	Kanamycin	K	30mcg	3	11.11	24	88.89
5.	Ciprofloxacin	CIP	5mcg	16	59.25	11	59.26
6.	Tetracycline	TE	10mcg	5	18.51	22	81.49
7.	Gentamicin	GEN	10mcg	7	25.92	20	74.08
8.	Pencillin-G	P	10 units	9	33.33	18	66.67
9.	Enrofloxacin	EX	10mcg	18	66.66	9	33.34
10.	Streptomycin	S	25mcg	4	14.81	23	85.19

Table 4: Effect of the treatment on the elimination of *S. aureus* from the milk of investigated cows in particular groups

CFU of *S. aureus* per 1 ml of milk									
Before treatment			7 days after treatment			21 days after treatment		35 days after treatment	
Group	0	1-104 >104	0	1-104	>104	0	1-104 >104	0	1-104 >104
Number of cows (%)									
Antibiotic	0	6(66.7) 3(33.3)	6(66.7) 3(33.3)		0 (0)	3(33.3) 5(55.6)	1(11.1)	3(33.3) 5(55.6)	1(11.1)
Autovaccine	0	4(44.4) 5(55.6)	3(33.3) 5(55.6)		1(11.1)	5(55.6) 4(44.4)	0(0)	6(66.7) 3(33.3)	0(0)
Antibiotic/Autovaccine	0	3(66.7) 6(33.3)	9(100) 0(0)		0(0)	9(100) 0(0)	0(0)	9(100) 0(0)	0(0)
$\chi 2$ test	$\chi 2$ =2.07; P = 0.72		$\chi 2$ =9.75; P =0.04			$\chi 2$ =9.96; P=0.04		$\chi 2$=9.75; P=0.04	

Fig 1*: In-vitro* antibiotic sensitivity test of *S. aureus* isolates -Therapeutic areas

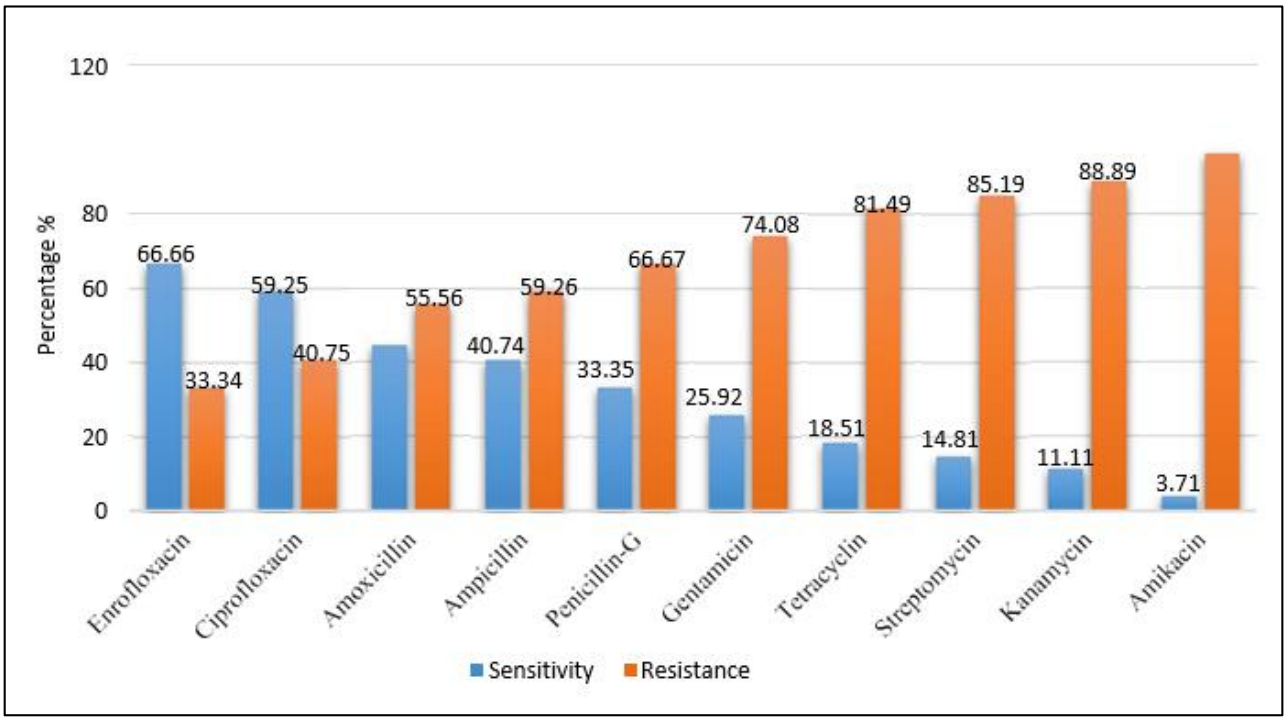

Fig 2: *In-vitro* antibiotic sensitivity patterns of *S. aureus* isolates -Therapeutic areas

Fig 3: *S. aureus* autovaccine safety test in mice

Fig 4: Farm trial of *S. aureus* autovaccine therapy at LFC, CVSc, Tirupati

Fig 5: Field trial of *S. aureus* autovaccine therapy at Sorakayalapalem

Fig 6: Field trial of *S. aureus* autovaccine therapy at Perur

Conclusion

In conclusion, the present study revealed that enrofloxacin is the drug of choice based on antibiogram of isolated strains of *S. aureus* from mastitis cases. Further, it was observed that *S. aureus* specific auto vaccine administered with enrofloxacin, an antibiotic of choice in the most effective method in the treatment of mastitis cased by S. aureus. Hence, *S, aureus* specific auto vaccine could be used effectively along with antibiotic for treating mastitis cases.

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