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Public Health risk of antibiotic resistant phenotypes and molecular confirmation of *staphylococcus aureus* isolated from bovine raw milk of South Gujarat, India

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Abstract
Background: Food poisoning health risk due to the consumption of contaminated food and antibiotic-resistant bacteria is currently a serious threat of public health.
Aims: This study aimed to determine the occurrence and antibiogram of *Staphylococcus aureus* isolated from bovine raw and bulk milk samples from pari-urban of Navsari and Surat, Gujarat.
Methods: A total of 120 samples comprising of fresh raw milk, cattle and buffalo pooled and mix bulk milk were collected standard microbiological procedures. Moreover, we evaluated 17 different antimicrobial agents on *S. aureus* for susceptibility and resistant by using D-test.
Results: Out of the 120 milk samples examined, 40 *S. aureus* were isolated showed a prevalence of 33.33% and all isolates were successfully amplified *16S rRNA* gene-based PCR. Antimicrobial sensitivity test of conformed all 40 isolates indicated phenotypes were completely to moderately resistance to Penicillin G followed by Cefixime, Cephotaxime, Methicillin and Oxytetracycline and other antibiotics were observed significance high susceptibility to Tylosin followed by Ampicillin, Gentamicin, Tetracycline, Chloramphenicol, Ciprofloxacin. All isolates showed a MAR index ranging between 0.06 to 0.47, after performing the correction for pairs of variables with a statistically significant Pearson correlation coefficient showed highest correlation of compounds from same class to other class and high correlation were yielded for aminoglycosides, quinolones and beta-lactams.
Conclusion: This study indicated the potential misuse of antibiotic and public health risks due to staphylococcal food poisoning were conditions congenial supports the bacterial growth.

Keywords: Milk, *S. aureus*, antibiotic resistant phenotypes, MAR index, 16S rRNA

Introduction
Zoonoses are infectious diseases that naturally be transmitted between animals and humans. The severity of these diseases in humans may vary according to the main origin of the infection (EFSA, 2008). Raw milk may contain pathogenic microorganisms and play a vital role in the transmission of these pathogenic bacteria to humans. Among the most isolated pathogenic bacteria in milk that cause disease were *Salmonella, Brucella, Listeria, Escherichia coli,* and *Staphylococcus aureus* (Vahedi *et al.,* 2013) [47].
Urban and peri-urban dairy production plays an important role in fulfilling the increasing demand of liquid milk and its products in developing countries. Milk content all essential nutrient for human food, which makes it perishable were support the growth of the microorganisms (Ajmal *et al.,* 2015) [2]. Recently, due to increase in world population, liquid milk demand has been increased tremendously (FAO, 2018) [13]. Foodborne infection risk is low in the countries where pasteurization is applied to most milk and its products, but there exists a risk with raw milk and its products made with raw milk. *S. aureus* also constitutes a threat to public health due to food safety and antibiotic usage issues and the potential for bidirectional transmission of strains between humans and dairy animals (Rainard *et al.,* 2018) [38]. *Staphylococcus aureus* has long been globally seen as normal colonizers of the as normal flora of the skin, axilla, and anterior nares of man and animals. Infections due to staphylococci are of major importance to veterinary and human medicine. Mortality associated with severe *S. aureus* infections in the developing world far exceeds that in developed countries. The epidemiology of this microorganism in animals has gained interest in the last few years back due to the increase of infectious processes caused by this pathogen (Jiménez *et al.,* 2013) [23] their increasing evidenced zoonotic potentials in people that are in direct contact with these animals as well as in their relations (Lozano *et al.,* 2016) [28]. The presence of *S. aureus* in milk and involvement of this pathogen in clinical and subclinical mastitis of dairy animals

(Seyoum *et al.,* 2018) [41] and demonstration of *S. aureus* in foods and food products like meat, dairy, eggs, chickens in different parts of India (Hachemi *et al.,* 2019) [18] and consumers preference ready-to-eat popular food sold in market favors to insure the health of consumers.

Antimicrobial resistance has been found even in antibiotic pressure is not exist in previously unexplored environments and it has been demonstrated that food can serve as a vehicle for transmission of multiple therapeutics agents resistance *S. aureus* to the human population (Founou *et al.,* 2020) [17]. The various emerging strains can be differentiated by antibiotic susceptibility, resistance phenotypic determinants virulence factors and genotyping (Mzee *et al.,* 2020) [31]. The number of research focused on the multiple antibiotic resistance problem of *S. aureus* has grown globally, in the last decade suggested that this problem is increasing especially in India. The development of multiple antibiotic-resistant bacteria due to indiscriminate use of antibiotics in animals and poultry production is well authenticated for pathogenic bacteria (Arenas *et al.,* 2017) [6]. The real extent of the burden of antimicrobial resistance of *S. aureus* is currently unknown where intensive surveillance of drug resistance is only carried out in a few countries (Shrivastava *et al.,* 2018) [43]. Identical elements of antibiotic-resistant genes found in bacteria that affect both animals and humans have shown the role of raw foods in the dissemination of these resistance genes through the food chains and occupational contact with livestock (Papadopoulos *et al.,* 2018, Patel *et al*., 2022) [35, 26]. Indeed, the comparison between human isolates and animal-derived isolates was performed also in other studies (Kadlec *et al*., 2019) [25] suggesting that food, after handling and processing, could represent a source of human infection, and for food operators a source of food contamination. Keeping in view of the public health significance, this study was conducted with the aim of isolating, characterizing, determining the antibiotic resistant phenotypes and molecular identification of *S. aureus* from bovine raw and bulk milk samples in parts of Navsari and Surat, Gujarat.

Materials and Methods

Study area

The present study was conducted in part of Navsari and Surat District of South Gujarat Province, India. Both district of province of Gujarat is most popular for dairy production, bordered to the east by Madhya Pradesh and to the south by Maharashtra. These parts of the state is semi dry to low humid and gradually present a high rainfall.

Sample collection

For the study and sampling purpose, the simple random sampling method was used. Total 120 milk samples were collected where 60 samples were raw pooled (mixed cattle and buffalo, cattle pooled, and buffalo pooled) milk and 60 samples from individual animal after treatment with specific antibiotics through veterinary doctor from various peri-urban and rural areas of district Surat and Navsari from November 2018 to January 2019. The bulk fresh milk samples were collected after the milking and have been pooled and collected in sterile milk collection falcon tube to prevent spillage and cross contamination.

Isolation and identification of *S. aureus*

The samples were processed to isolate and identify *S. aureus* as per the standard methods described in Bacteriological Analytical Manual (BAM) (FDA and USDA, 2016) [15] and Peacock (2010) [37] with certain modification.

The milk samples were thoroughly mixed aseptically before processing and from each collection tube 1 ml milk was drawn with the help of sterile pipette and transferred into 10% marked 9 ml enrichment Buffer Peptone water in separate test tube. The test tubes to obtained enrich culture were mixed and incubate at 37o C for 24 hours. All samples were inoculated with the aid of a sterile wire loop onto the surface of prepared Baird-Parker agar (Himedia, Mumbai) supplemented with 5% egg yolk tellurite emulsion (Baird-Parker, 1962). Discrete colonies were further sub-cultured on to freshly prepared plates of Mannitol Salt Agar (MSA) medium for selective isolation of *S. aureus* and nutrient agar plates for biochemical tests and identification (Njage *et al.,* 2013) [33]. *S. aureus* ATCC 25923 strain was used as a positive control. Presumptive morphological identification of the colonies was done by observing their individual appearance on the selective media that was used for the isolation and Gram reaction. The conventional biochemical tests carried out to identify the suspected *S. aureus* colonies were motility test, voges proskauer test, catalase test, slide coagulase test, haemolysis on blood agar and also performed to detect production of acid and gas from Sucrose, Lactose, D-(+)-Maltose, D-(+)-Xylose, D-Mannitol in 1% (w/v) Andrade peptone water with inverted Durham tubes.

Determination of the antibiogram of the isolates

The antibiogram of the isolates was determined using the Kirby-Bauer agar disc diffusion method as described by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2018) [9]. Total 17 antibiotic incorporated discs were chosen according to their mode of action and their use in clinical therapy. Those that inhibit cell wall synthesis included Penicillin, Vancomycin, Methicillin, Cefixime, Cephotaxime, Ceftriaxone and Ampicillin. Those that inhibit protein synthesis included Tetracycline, Oxytetracycline, Gentamicin, Kanamycin, Tylosin, Chloramphenicol and Streptomycin. Those that inhibit nucleic acid synthesis included Enrofloxacin, Ciprofloxacin and Ofloxacin were tested for quality with ATCC - 25922 *E. coli* and ATCC - 25923 *Staphylococcus aureus* as per guidelines of CLSI (2018) [9]. An entire of 0.5 McFarland and concentrations of bacteria was applied for this goal.

Determination of multiple antibiotics resistance (MAR) index

The multiple antibiotics resistance (MAR) index was determined for each of the isolate using the formula: MARI = x/y, where "X" is the number of antibiotics to which the isolate display resistance and "Y" is the total number of antibiotics to which the test organism had been evaluated for sensitivity (Tula *et al.,* 2013) [46].

Molecular Characterization of the isolates

The bacterium isolated from samples were conferment by targeting *16S rDNA* gene of *S. aureus* species using PCR. For the molecular level identification of the isolate's, isolated colonies were cultured in Luria-Bertani broth and incubated at 37ºC for 24 hrs. Then DNA of 24 h old cultures were extracted using the mericon DNA Bacteria plus Kit (Qiagen, Germany) with some modifications from the manufacturer's recommendations. Fragments of the genes of interest were amplified using standard PCR protocol the sequence of first

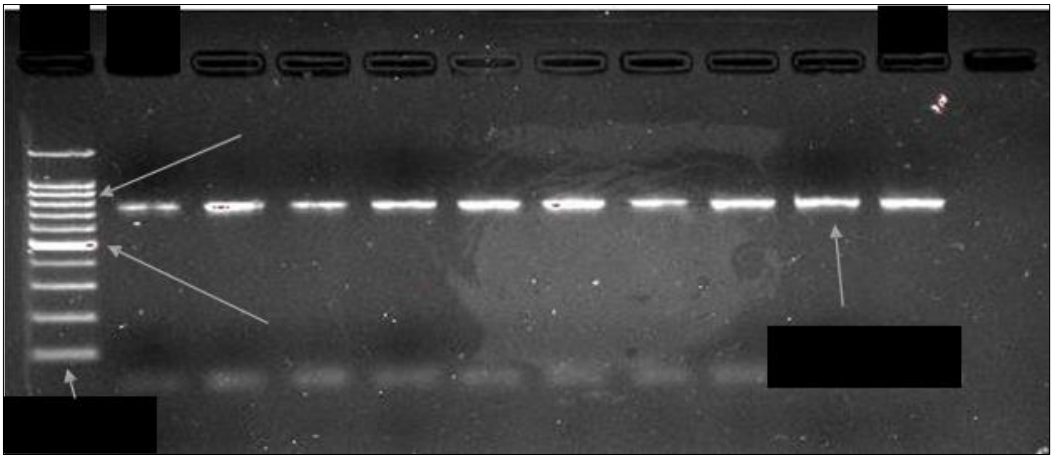
set of primers F - AACTCTGTTATTAGGGAAGAACA and R – CCACCTTCCTCCGGTTTGTCACC obtained from published work (Maes *et al.,* 2002) [29] was specific for *16S rDNA* gene of the genus *S. aureus* species were commercially synthesized from Eurofins Genomic, India.

Statistics Analysis

The results were analyzed statistically using the SPSS software for Windows (version 20.0; SPSS Inc., Chicago, IL, USA). Statistical significance of the differences in resistance was evaluated using the statistical analysis system (Hosseinzadeh and Sei, 2014) [20]. The Pearson chi- square test was used to determine the statistically significant difference between the occurrence of *S. aureus* and the different sample types and the occurrence of *S. aureus* in fresh and pooled milk in different sampling areas.

Results

In the present study, a total of 40/120 (33.33%) positive isolates of *Staphylococcus aureus* obtained from 120 raw milk samples screened (Table 1). The isolates were molecular conformed with gene-based PCR assay and conventional assay were staining exhibited clusters of grape appearance and gram-positive cocci with the ability to ferment mannitol and produce golden yellow colour colonies on Mannitol salt agar (MSA) and jet black colour colony in BP medium. Further biochemical characterization showed positive reaction with catalase on 3% hydrogen peroxide, slide coagulase test using rabbit coagulase plasma, Motility test, Voges Proskauer test, beta haemolysis on blood agar and various sugar fermentation test (Himedia, India). The molecular confirmation of *S. aureus* isolates with *16S rDNA* gene amplified a 750 bp specific amplicon size product (Figure: 1). During the present study, all 40 *S. aureus* isolates were found variably resistant to susceptibility the antibiotics tested (Figure: 3 and Table: 3). Among the 40 *S. aureus* isolates maximum resistance was observed for completely resistant to moderately resistance penicillin (100%) followed by cefixime (87.50%), cephotaxime (75%), Methicillin and oxytetracycline (57.50%). While practicing the veterinary profession were sensitivity of the *S. aureus* isolates towards tylosin (100%) followed by ampicillin (95%), gentamicin (77.50%), tetracycline (70%), chloramphenicol and ciprofloxacin (67.50%), kanamycin (65%), vancomycin, ofloxacin and enrofloxacin (60%), streptomycin and ceftriaxone (52.50%). PCR was selected specific and valuable method for detection of *S. aureus* due to its accuracy and rapidness, because rapid and proper detection of pathogen is necessary. Identification of *S. aureus* done by using amplification of DNA of reference strain of *S. aureus* and 40 of isolates from 120 samples showed amplification of the 750 bp region of the *16S rRNA* gene for *S. aureus*.



Lane 1: Molecular marker, 100 plus bp,
Lane 2- PC: Positive control of ATCC 25923 *Staphylococcus aureus* DNA
Lane 3 to 11: *S. aureus* isolates carrying *16s rRNA* gene (750 bp)
Lane 12 - NC: Negative control (reagents with primers without DNAs),

Fig 1: Specificity of PCR assay of DNA from *Staphylococcus aureus* sample:

Table 1: The occurrence of *S. aureus* in relation to the type of milk samples collected from parts of South Gujarat

Type of Milk Sample	No. examined	No. positive	Percentage (%)	*X2*	*p*-value
Cow pooled Milk	20	7	35	0.455	0.994
Buffalo Pooled Milk	20	7	35		
Mix Bulk milk	20	7	35		
Individual raw milk	60	19	31.67		
Cattle	30	8	26.67		
Buffalo	30	11	36.67		

Table 2: The prevalence of *S. aureus* in raw and bulk milk in relation to the local government areas sampled of South Gujarat

Local Government Areas	No. examined	No. positive	Percentage (%)	*X2*	*p*-value
Navsari	49	13	26.53	1.489	0.685
Jalalpor	53	22	41.51		
Gandevi	6	2	33.33		
Surat	12	3	25		
Total	120	40	33.33		

Discussion

The occurrence of *S. aureus* (33.33%) in the study area is an indication of defective or absence of public health measures and poor sanitary habits among the people that are concerned with milking, milk handling, and transportation as these have been documented to be factors that predisposes milk to contamination with pathogens (Akram *et al.,* 2013) [3]. Table: 2 shows the prevalence (%) of *S. aureus* obtained from fresh and pooled milk in the four sampling areas selected for this study. Out of the 120 samples collected during the study, 40 (33.33%) were found to be contaminated with *S. aureus*. Reddy *et al.* (2015) [40] and Ramya *et al.* (2017) [39] who have also subjected their bacterium of *staphylococci* with same biochemical tests and observed similar type of reactions.

The percentage occurrence of *S. aureus* in bovine raw and pooled milk samples was recorded in this study was 33.33%, which was higher than below study that 8.7%, 12.14% and 25.53% recorded by Okpo *et al.* (2016) [34], Usman and Mustapha (2016) and Jahan *et al.* (2015) [22], respectively. Sudhanthiramani *et al.* (2015) [44] and Deepake *et al.* (2020) [10] reported that prevalence of 39.09% and 34.49% coagulase-positive *S. aureus* from the milk samples which is in accordance with our study, whereas Bhati *et al.* (2018) [8] reported 54.31% *S. aureus* isolates confirmed from mastitis milk samples, udder and milkers' hands by *23s rRNA* based PCR in Rajasthan, India. Akriti *et al.* (2019) [4] documents the prevalence of *S. aureus* as 66.66% (40/60) in the cattle milk samples collected from Vallabhnagar tehsil of Udaipur district. Variation in the occurrence of *S. aureus* may be attributed to various factors like sampling design, study location and methodology adopted. Table: 2 shows the occurrence of *S. aureus* in relation to the type of milk samples collected from parts of South Gujarat, India.

Statistically significant difference ($p>0.05$) was found using chi-square in the occurrence of *S. aureus* in fresh and pooled milk with respect to the different sample types collected during this study, indicating that, the milk samples might have been exposed to the same levels of contamination. The occurrence of *S. aureus* in fresh and bulk milk in this study may be attributed to the presence of sub-clinical mastitis in the milked cows, poor sanitary practices during milking, and unclean milking utensils. This is of health risk and public health significance since it is a commonly recovered pathogen in outbreaks of food poisoning attributed to dairy products. Proper heat treatment and refrigeration can minimize the chances of contamination with *S. aureus* (Junaidu *et al.,* 2011) [24].

Multi drug resistance is now the norm among the Gram-Positive bacteria like *S. aureus* is perhaps the pathogen of concern because of its intrinsic virulence, its ability to poses a problem of public health concern and effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become difficult. In the present study, *S. aureus* isolates have showed highest resistance to penicillin (100%) which agreed with the reports of Khakpoor *et al.* (2011) [26], Thaker *et al.* (2013) [45] and Jahan *et al.* (2015) [22] who have recorded 100% resistance to penicillin among *S. aureus* isolates. Slightly lower percentage of resistance to penicillin than the findings of this investigation was observed from the findings of Elemo *et al.* (2017) [11], Can *et al.* (2017), Yadav (2018) [48] and Fawzy *et al.* (2017) [14] who have reported 87.3%, 81.81%, 82.23% and 73.6% of resistance respectively.

In this investigation resistance to tetracycline was found to be a 10% among the isolates of *S. aureus* which agreed with the report Jackson *et al.* (2013) [21], Shamila-Syuhada *et al.* (2016) [42] and Feng *et al.* (2016) [16] who found 25%, 5% and 15.91% of resistance Tetracycline among the isolates of *S. aureus*, respectively. Intermediate resistance to gentamicin in the present study by the isolates of *S. aureus* was 22.50% which agreed with the reports of Momtaz *et al.* (2013) [31] and Mashouf *et al.* (2015) [30] who found 29.26% and 27.6% of resistance in the isolates of *S. aureus* respectively. Lower resistance to gentamycin compared to the present findings was observed by Thaker *et al.* (2013) [45], Tigabu *et al.* (2015), Feng *et al.* (2016) [16] and Wang *et al.* (2018) who have reported 10%, 2.8%, 9.09%, and 1% respectively.

The isolates of *S. aureus* have shown resistance to ciprofloxacin, and it was found as 7.5% which agreed with the report of Ammar *et al.* (2016) [5], K Harish *et al.* (2019) [19] and Awad *et al.* (2017) [7] who have observed 10%, 11.5% and 14.3% resistance in the isolates of *S. aureus.*

Higher resistance compared to the present finding was observed by Pati and Mukherjee (2016), Wang *et al.* (2018) and Yadav (2018) [48] who have reported 37%, and 18.8% and 42.1% of resistance respectively among the isolates of *S. aureus*. This finding is not surprising because, outside the animal hospital environment, people have easy access to various antibiotics at any drug store without any prescription from qualified personnel.

26 antibiotic resistance phenotypes were obtained, all from the multiple resistance types with varying combinations of one to eight antibiotics. This finding is in consonance Chaalal *et al.* (2016) who reported cases of multidrug resistance among *S. aureus* isolated from dairy products, respectively. 100% of the *S. aureus* isolates obtained in this study had a MAR index of 1.6 and above. MAR index gives an indirect suggestion of the probable source of an organism (Adesokan *et al.,* 2013) [1].

In this study, highest Pearson correlation coefficient characterized the compounds of the same class to other compounds of the same class (i.e. resistance to one beta-lactam is correlated to resistance to another beta-lactam). As expected, high correlation coefficients for aminoglycosides (>0.54), quinolones (>0.69) and beta-lactams (1.00) were yielded. The shading in Table 8 also clearly indicates the pairwise relationship between group of aminoglycosides, penicillin, quinolones, tetracyclines and cephalosporins (3rd and 4th generation). Such association is reflected by the well-known occurrence of multi-drug resistance in staphylococcal clones (Lindsay *et al.* 2012) [27].

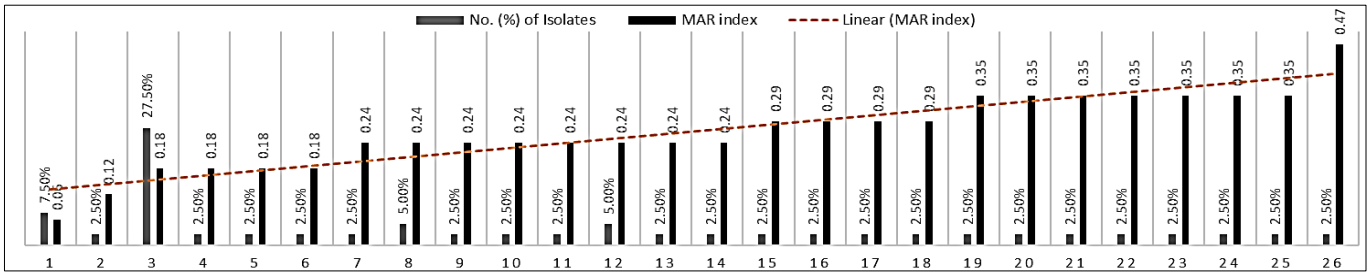


Fig 2: Multiple Antibiotic Residence (MAR) index of s. aureus isolated from fresh raw and pooled milk in parts of Navratri and Surat, Gujarat

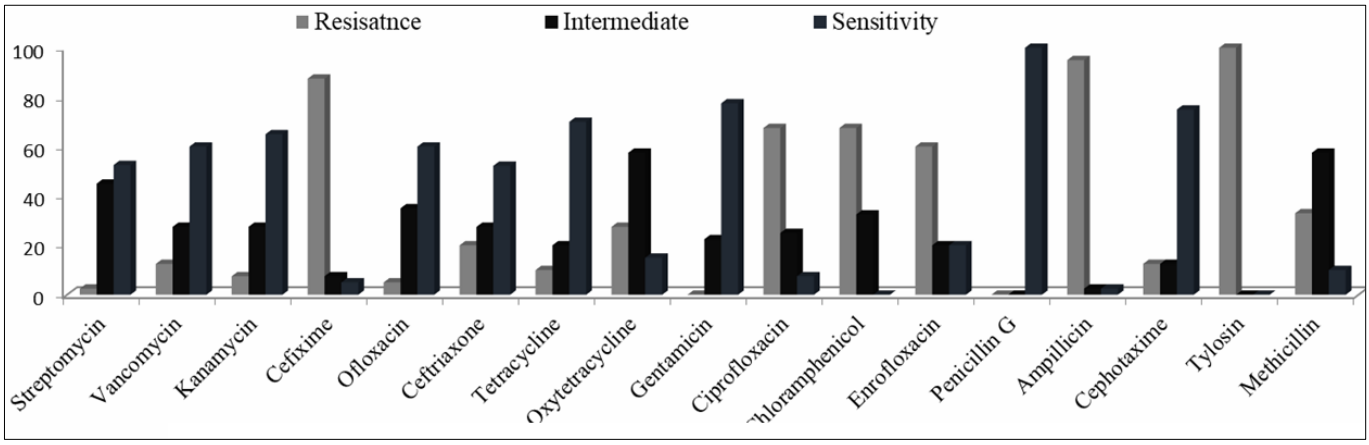


Fig 3: Showing antibiotic resistance and susceptibility (%) pattern of *S. aureus*

Table 3: Interpretation chart for antibiotic sensitivity/resistance patterns of isolates

Sr. No.	Antibiotic discs	Discs concentration (mcg)	Diameter of zone of inhibition in mm			N = 40 isolates		
			Sensitive	Intermediate resistant	Resistant	S (%)	I (%)	R (%)
1	Streptomycin (S)	15	≥15	12-14	≤11	52.50	45.00	2.50
2	Vancomycin (VA)	30	≥17	15-16	≤14	60.00	27.50	12.50
3	Kanamycin (K)	30	≥18	14-17	≤13	65.00	27.50	7.50
4	Cefixime (CFM)	5	≥19	16-18	≤15	5.00	7.50	87.50
5	Ofloxacin (OF)	30	≥26	16-25	≤15	60.00	35.00	5.00
6	Ceftriaxone (CTR)	30	≥23	20-22	≤19	52.50	27.50	20.00
7	Tetracycline (TE)	30	≥15	13-14	≤11	70.00	20.00	10.00
8	Oxytetracycline (OTC)	30	≥26	16-25	≤15	15.00	57.50	27.50
9	Gentamicin (GEN)	10	≥15	13-14	≤12	77.50	22.50	0.00
10	Ciprofloxacin (CIP)	5	≥21	16-20	≤15	67.50	25.00	7.50
11	Chloramphenicol (C)	30	≥18	13-17	≤12	67.50	32.50	0.00
12	Enrofloxacin (EN)	10	≥21	17-20	≤16	60.00	20.00	20.00
13	Penicillin G (P)	10 units	≥29	-	≤28	0.00	0.00	100
14	Ampillicin (AMP)	10	≥17	14-16	≤13	95.00	2.50	2.50
15	Cephotaxime (CTX)	30	≥26	23-25	≤22	12.50	12.50	75.00
16	Tylosin (TL)	15	-	-	-	100	0.00	0.00
17	Methicillin (MET)	10	≥14	10-13	≤9	33.00	57.50	10.00
Where: S – Sensitive, I – Intermediate resistant and R - Resistant								

Table 4. The antibiotic resistance patterns of *S. aureus* isolated from raw and pooled milk samples in parts of South Gujarat, India.

Sr. No.	No. of antibiotics	Resistance pattern	No. (%) of Isolates	MAR index	Local Government Area			
					N	J	S	G
1	1	P	3 (7.50%)	0.06	2	1	-	-
2	2	P, CFM	1 (2.50%)	0.12	-	-	-	1
3	3	P, CFM, CTX	11 (27.50%)	0.18	4	6	1	-
4	3	P, VA, CFM	1 (2.50%)	0.18	1	-	-	-
5	3	P, CFM, OTC	1 (2.50%)	0.18	1	-	-	-
6	3	P, MET, OTC	1 (2.50%)	0.18	-	1	-	-
7	4	P, CFM, OTC, CIP	1 (2.50%)	0.24	1	-	-	-
8	4	P, CFM, CTR, CTX	2 (5.00%)	0.24	2	-	-	-
9	4	P, CTR, CTX, EN	1 (2.50%)	0.24	-	1	-	-
10	4	P, CFM, CTR, OTC	1 (2.50%)	0.24	-	1	-	-
11	4	P, MET, CFM, CTR	1 (2.50%)	0.24	-	1	-	-
12	4	P, CFM, CTX, OTC	2 (5.00%)	0.24	-	2	-	-

13	4	P, CFM, CTX, K	1 (2.50%)	0.24	-	1	-	-
14	4	P, MET,CFM, CTX	1 (2.50%)	0.24	-	1	-	-
15	5	P, CFM, CTX, TE, OTC	1 (2.50%)	0.29	-	-	-	1
16	5	P, CFM, CTX, EN, OTC	1 (2.50%)	0.29	1	-	-	-
17	5	P, VA, CFM, TE, EN	1 (2.50%)	0.29	1	-	-	-
18	5	P, MET, CFM, CTX, OTC	1 (2.50%)	0.29	-	1	-	-
19	6	P, CFM, CTX, AMP, OTC, EN	1 (2.50%)	0.35	-	1	-	-
20	6	P, CFM, VA, CTX, EN, CIP	1 (2.50%)	0.35	-	-	1	-
21	6	P, CFM, CTX, TE, K, S	1 (2.50%)	0.35	-	1	-	-
22	6	P, VA, CFM, CTR, CTX, EN	1 (2.50%)	0.35	-	1	-	-
23	6	P, CFM, CTR, CTX, CIP, OF	1 (2.50%)	0.35	-	1	-	-
24	6	P, CFM, CTX, OTC, CIP, OF	1 (2.50%)	0.35	-	1	-	-
25	6	P, CFM, CTR, CTX, K, S	1 (2.50%)	0.35	-	1	-	-
26	8	P, VA, CFM, CTR, CTX, OTC, TE, CIP	1 (2.50%)	0.47	-	1	-	-

P - Penicillin G, CFM - Cefixime, CTR – Ceftriaxone, CTX - Cephotaxime, MET - Methicillin, VA- Vancomycin, K-Kanamycin, S-Streptomycin, EN -, AMP - Ampicillin, CIP -
Ciprofloxacin, OF – Ofloxacin, OTC - Oxytetracycline, TE – Tetracycline N - Navsari, G - Gandevi, S - Surat, J - Jalalpor

Table 5: Pearson Correlation coefficient between antimicrobial susceptibility profiles of over 40 *S. aureus* isolate from bovine raw milk

	P	VA	MET	CFM	CTR	CTX	AMP	TE	OTC	GEN	K	TL	C	S	EN	CIP	OF
P	1.000																
VA	0.110	1.000															
MET	0.236	0.076	1.000														
CFM	-0.029	0.260	0.191	1.000													
CTR	0.529**	0.156	0.288	0.154	1.000												
CTX	0.284	-0.029	0.289	0.402*	0.423**	1.000											
AMP	0.284	-0.029	0.289	0.402*	0.423**	1.000**	1.000										
TE	0.061	0.161	0.348*	-0.044	-0.125	-0.038	-0.038	1.000									
OTC	0.012	0.070	0.348*	-0.074	-0.044	0.031	0.031	0.537**	1.000								
GEN	0.370*	0.310	0.093	0.329*	0.553**	0.354*	0.354*	0.090	0.053	1.000							
K	-0.191	0.103	0.427**	0.021	0.133	-0.102	-0.102	0.153	0.165	0.162	1.000						
TL	-0.051	-0.236	-0.050	-0.185	-0.045	-0.156	-0.156	-0.076	0.204	-0.031	0.216	1.000					
C	0.201	-0.179	0.222	-0.264	0.384*	0.052	0.052	0.241	0.291	0.083	0.252	0.046	1.000				
S	0.126	0.354*	0.537**	0.403**	0.274	0.257	0.257	0.400*	0.440**	0.322*	0.304	0.036	0.401*	1.000			
EN	0.017	0.293	0.272	0.417**	0.287	0.390*	0.390*	0.301	0.293	0.378*	0.125	-0.120	0.252	0.597**	1.000		
CIP	0.055	0.338*	0.347*	0.204	0.298	0.171	0.171	0.397*	0.336*	0.376*	0.359*	-0.065	0.333*	0.668**	0.622**	1.000	
OF	0.228	0.179	0.183	-0.183	0.317*	0.076	0.076	0.175	0.122	0.311	0.351*	0.155	0.226	0.138	0.354*	0.393*	1.000

All shaded cells report statistically significant correlations, P – Penicillin G, AMP - Ampicillin, VA - Vancomycin, MET - Methicillin, CFM - Cefixime, CTR - Ceftriaxone,
CTX - Cephotaxime, TE - Tetracycline, OTC - Oxytetracycline, GEN - Gentamicin, K - Kanamycin, TL - Tylosin, C – Chloramphenicol, S - Streptomycin, CIP - Ciprofloxacin, OF – Ofloxacin, EN – Enrofloxacin
**. Correlation is significant at the 0.01 level (2-tailed) *. Correlation is significant at the 0.05 level (2-tailed).

Conclusion

This study attempted to provide baseline information for the prevalence and antibiotics resistance of *S. aureus* in fresh raw and pooled milk from apparently healthy bovines in South Gujarat, Gujarat. Rapidly emerging multi-drug resistant of *S. aureus* pose a serious threat to public health and make treatment failure quite imminent, therefore Indian countries, including Gujarat, should consider having efficient control over misuse of antibiotics. The use of antibiotics in animal husbandry as growth promoters must be discouraged as this enhances antibiotics-resistance among *S. aureus.* Bringing awareness among the public about the harmful effects of multi drug resistant microflora is another important objective for the scientists to protect the humans from these super bugs.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Adesokan HK, Agada CA, Adetunji VO, Akanbi IM. Oxytetracycline and penicillin-G residues in cattle slaughtered in south-western Nigeria: Implications for livestock disease management and public health. Journal of the South African Veterinary Association. 2013;84(1):01-05.
2. Ajmal MM, Li CX, Aslam W. Current status of dairy industry in five districts of Punjab, Pakistan. J. econ. Sustain. Dev. 2015;6:19-28.
3. Akram N, Chaudhary AH, Ahmed S, Ghuman MA, Nawaz G, Hussain S. Isolation of bacteria from mastitis affected bovine milk and their antibiogram. European Journal of Veterinary Medicine. 2013;2(1):38-46.
4. Akriti Diwakar, Gaurav A. Prevalence and antibiotic resistance pattern of Staphylococcus aureus of dairy origin from Udaipur (Rajasthan) region. J. Entomol. Zool. Stud. 2019;7(4):1143-1145.
5. Ammar AM, Attia AM, Abd El-Hamid MI, El-Shorbagy IM, Abd El-Kader SA. Genetic basis of resistance waves among methicillin resistant Staphylococcus aureus isolates recovered from milk and meat products in Egypt. Cellular and Molecular Biology. 2016;62(10):7-15.
6. Arenas NE, Abril DA, Valencia P, Khandige S, Soto CY, Moreno-Melo V. Screening food-borne and zoonotic

pathogens associated with livestock practices in the Sumapaz region, Cundinamarca, Colombia. Tropical animal health and production. 2017;49(4):739-745.
7. Awad A, Ramadan H, Nasr S, Ateya A, Atwa S. Genetic Characterization, Antimicrobial Resistance Patterns and Virulence Determinants of Staphylococcus aureus Isolated form Bovine Mastitis. Pakistan journal of biological sciences. Pakistan Journal of Biological Sciences. 2017;20(6):298-305.
8. Bhati T, Kumar G, Khichar V, Kataria AK. Prevalence of Staphylococcus aureus isolated from mastitic milk, udder surfaces and milkers' hands from different farms in Bikaner, Rajasthan. Journal of Animal Research. 2018;8(5):867-872.
9. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; c2018.
10. Deepak SJ, Porteen K, Elango A, Kumar TMA, Babu RN, Sureshkannan S, *et al*. Occurrence of Methicillin Resistant Staphylococcus aureus from Bovine Raw Milk in Chennai. Journal of Animal Research. 2020;10(1):127-131.
11. Elemo KK, Sisay T, Shiferaw A, Fato MA. Prevalence, risk factors and multidrug resistance profile of Staphylococcus aureus isolated from bovine mastitis in selected dairy farms in and around Asella town, Arsi Zone, South Eastern Ethiopia. African Journal of Microbiology Research. 2017;11(45):1632-1642.
12. European Food Safety Authority (EFSA). Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal Escherichia coli and Enterococcus spp. from food animals. EFSA journal. 2008;6(4):141r.
13. FAO. Dairy market review. FAO. Rome; c2018. Available at: http://www.fao.org/3/I9210EN/i9210en.pdf (accessed 17 Sep 2018).
14. Fawzy R, Samy AA, Salam HS, Khairy EA, Koraney AA. Polymerase chain reaction detection of genes responsible for multiple antibiotic resistance Staphylococcus aureus isolated from food of animal origin in Egypt. Veterinary World. 2017;10(10):1205.
15. FDA (Food and Drug administration). BAM: *Staphylococcus aureus.* Bacteriological Analytical Manual; c2016.
16. Feng YANG, Qi WANG, Wang XR, Ling WANG, LI XP, LUO JY, *et al*. Genetic characterization of antimicrobial resistance in Staphylococcus aureus isolated from bovine mastitis cases in Northwest China. Journal of integrative agriculture. 2016;15(12):2842-2847.
17. Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PloS one. 2017;12(12):e0189621.
18. Hachemi A, Zenia S, Denia MF, Guessoum M, Hachemi MM, Ait-Oudhia K. Epidemiological study of sausage in Algeria: Prevalence, quality assessment, and antibiotic resistance of Staphylococcus aureus isolates and the risk factors associated with consumer habits affecting foodborne poisoning. Veterinary world. 2019;12(8):1240.
19. Harish K, Babu AJ, Rao TM, Sreedevi B. A study on the antimicrobial resistant patterns and molecular characterization of Staphylococcus aureus isolated from milk. The Pharma Innovation Journal. 2019;8(8):160-168.
20. Hosseinzadeh S, Dastmalchi Saei H. Staphylococcal species associated with bovine mastitis in the North West of Iran: emerging of coagulase-negative staphylococci. International Journal of Veterinary Science and Medicine. 2014;2(1):27-34.
21. Jackson CR, Davis JA, Barrett JB. Prevalence and characterization of methicillin-resistant Staphylococcus aureus isolates from retail meat and humans in Georgia. Journal of clinical microbiology. 2013;51(4):1199-1207.
22. Jahan M, Rahman M, Parvej MS, Chowdhury SMZH, Haque ME, Talukder MAK, *et al*. Isolation and characterization of Staphylococcus aureus from raw cow milk in Bangladesh. Journal of Advanced Veterinary and Animal Research. 2015;2(1):49-55.
23. Jiménez JN, Ocampo AM, Vanegas JM, Rodriguez EA, Mediavilla JR, Chen L, *et al*. A comparison of methicillin-resistant and methicillin- susceptible Staphylococcus aureus reveals no clinical and epidemiological but molecular differences. International Journal of Medical Microbiology. 2013;303(2):76-83.
24. Junaidu AU, Salihu MD, Tambuwal FM, Magaji AA, Jaafaru S. Prevalence of mastitis in lactating cows in some selected commercial dairy farms in Sokoto metropolis. Adv Appl Sci Res. 2011;2(2):290-294.
25. Kadlec K, Entorf M, Peters T. Occurrence and characteristics of livestock- associated methicillin-resistant Staphylococcus aureus in quarter milk samples from dairy cows in Germany. Frontiers in microbiology. 2019;10:1295.
26. Khakpoor M, Safarmashaei S, Jafary R. Study of milk extracted from cows related to Staphylococcus aureus by culturing and PCR. Global Veterinaria. 2011;7(6):572-575.
27. Lindsay JA, Knight GM, Budd EL, McCarthy AJ. Shuffling of mobile genetic elements (MGEs) in successful healthcare-associated MRSA (HA-MRSA). Mobile genetic elements. 2012;2(5):239-243.
28. Lozano C, Gharsa H, Ben Slama K, Zarazaga M, Torres C. Staphylococcus aureus in animals and food: Methicillin resistance, prevalence and population structure. A review in the African continent. Microorganisms. 2016;4(1), 12.
29. Maes N, Magdalena J, Rottiers S, De Gheldre Y, Struelens MJ. Evaluation of a triplex PCR assay to discriminate Staphylococcus aureus from coagulase-negative staphylococci and determine methicillin resistance from blood cultures. Journal of Clinical Microbiology. 2002;40(4):1514-1517.
30. Mashouf RY, Hosseini SM, Mousavi SM, Arabestani MR. Prevalence of enterotoxin genes and antibacterial susceptibility pattern of Staphylococcus aureus strains isolated from animal originated foods in West of Iran. Oman medical journal. 2015;30(4):283.
31. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A, Momeni M. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. Journal of Applied Poultry Research. 2013;22(4):913-921.
32. Mzee T, Kazimoto T, Madata J, Masalu R, Bischoff M, Matee M, *et al*. Prevalence, antimicrobial susceptibility and genotypic characteristics of Staphylococcus aureus in

Tanzania: a systematic review; c2020.
33. Njage PMK, Dolci S, Jans C, Wangoh J, Lacroix C, Meile L. Phenotypic and genotypic antibiotic resistance patterns of Staphylococcus aureus from raw and spontaneously fermented camel milk. European Journal of Nutrition & Food Safety, 2013, 87- 98.
34. Okpo NO, Abdullahi IO, Whong CMZ, Ameh JB. Occurrence and antibiogram of Staphylococcus aureus in dairy products consumed in parts of Kaduna State, Nigeria. Bayero Journal of Pure and Applied Sciences. 2016;9(2):225-229.
35. Papadopoulos P, Papadopoulos T, Angelidis AS, Boukouvala E, Zdragas A, Papa A, *et al*. Prevalence of Staphylococcus aureus and of methicillin-resistant S. aureus (MRSA) along the production chain of dairy products in north-western Greece. Food microbiology. 2018;69:43-50.
36. Patel NM, Kumar R, Savalia CV, Kalyani IH. Public Health Risk Assessment of Fluoroquinolones, Gentamicin and Tetracycline Residues in Bovine Milk of Peri-Urban Area of Surat and Navsari of South Gujarat, India. Agricultural Reviews. 2018;5:1-6.
37. Peacock SJ. Staphylococcus. Topley & Wilson's Microbiology and Microbial Infections; c2010.
38. Rainard P, Foucras G, Fitzgerald JR, Watts JL, Koop G, Middleton JR. Knowledge gaps and research priorities in Staphylococcus aureus mastitis control. Transboundary and emerging diseases. 2018;65:149-165.
39. Ramya P, Rajeshukumar S, Sujatha S, Venkateswara RL. Prevalence of Staphylococcus aureus in raw milk samples. International Journal of Chemical Studies. 2017;5(5):1351-1353.
40. Reddy MS, Babu AJ, Ramya P, Swetha CS. Molecular characterization of Methicillin resistant *Staphylococcus aureus* from goats, pigs and their handlers. International Journal of Microbiology Research. 2015;7(3):648-655.
41. Seyoum B, Kefyalew H, Abera B, Abdela N. Prevalence, risk factors and antimicrobial susceptibility test of Staphylococcus aureus in Bovine cross breed mastitic milk in and around Asella town, Oromia regional state, southern Ethiopia. Acta tropica. 2018;177:32-36.
42. Shamila-Syuhada AK, Rusul G, Wan-Nadiah WA, Chuah LO. Prevalence and Antibiotics Resistance of Staphylococcus aureus Isolates Isolated from Raw Milk Obtained from Small-Scale Dairy Farms in Penang, Malaysia. Pakistan Veterinary Journal. 2016;36(1):98-102.
43. Shrivastava SR, Shrivastava PS, Ramasamy J. World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Journal of Medical Society. 2018;32(1):76.
44. Sudhanthiramani S, Swetha CS, Bharathy S. Prevalence of antibiotic resistant Staphylococcus aureus from raw milk samples collected from the local vendors in the region of Tirupathi, India. Veterinary World. 2015;8(4):478.
45. Thaker HC, Brahmbhatt MN, Nayak JB, Thaker HC. Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand, Gujarat. Veterinary World. 2013;6(1):10-13.
46. Tula MY, Azih AV, Okojie RO. Antimicrobial susceptibility pattern and plasmid-mediated antibacterial resistance in Staphylococcus aureus and Coagulasenegative Staphylococci (CoNS). American Journal of Research Communication. 2013;1(9):149-166.
47. Vahedi M, Nasrolahei M, Sharif M, Mirabi AM. Bactériological study of raw and unexpired pasteurized cow's milk collected at dairy farms and supermarkets in Sari city in 2011. J. Prev. Med. Hyg. 2013;54(2):120-123.
48. Yadav MM. Prevalence of Staphylococcus aureus in lactating cows with subclinical mastitis and their antibiogram in organized dairy farm, Maharashtra, India. Int. J. Curr. Microbiol. App. Sci. 2018;7(3):3674-3680.