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Research Article

High Prevalence of Multidrug Resistant *Staphylococcus aureus* from Buffalo Beef Sold at Retail Butcheries in Northern India

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Abstract

Staphylococcus aureus is a Gram positive cocci bacterium causes food poisoning in human population due to consumption of contaminated food. The bacterium may be part of the normal human microbiota which can cause wide range of diseases from skin and soft-tissue infections to severe invasive disease such as infective endocarditis, osteomyelitis, and toxic shock syndrome. In past, the antibiotic resistance of *S. aureus* has increased manifolds due to the bacterial evolution and antibiotic exploitation of antibiotics. Therefore, screening of antibiotic resistance in *S. aureus* time to time can be useful enormously for the treating *S. aureus* associated infections. Hence, this study was intended to investigate the status of antibiotic resistance of *S. aureus* in beef of buffalo origin. Fifty raw beef samples were randomly collected from different retail butcheries shops in Bareilly city, northern India and determined the load and prevalence of *S. aureus*. The mean values of *S. aureus* count were observed from $\log_{10}$ 1.0 cfu/g to log 3.6 cfu/g. Beef samples were also screened for the prevalence (36.0%) of the *S. aureus* following cultural methods of BAM/CFSAN and isolated eighteen *S. aureus* isolates. All isolates were confirmed by polymerase chain reaction (aPCR) targeting *nuclease* (*nuc*) gene. The isolates were scrutinized for antibiotic resistance. The utmost resistance were showed for penicillin (72.2%) and ampicillin (44.4%). The high prevalence (94.4%) of multidrug resistance (MDR) was monitored in *S. aureus* isolates. High prevalence of buffalo beef with antimicrobial-resistant *S. aureus* exhibits an alarming situation and obliges for specific utilization of antibiotics in buffaloes in the study area.

Keywords: *S. aureus*; Beef; *nuc* Gene; Multidrug Resistance (MDR)

Abbreviations

CDC: Centers for Disease Control and Prevention; USFDA: United States Food and Drug Administration; BAM: Bacteriological Analytical Manual; CFSAN: Center for Food Safety and Applied Nutrition; cfu: Colony Forming Unit; PCR-Polymerase Chain Reaction; EDTA: Ethylenediaminetetraacetic Acid; SDS; Sodium Dodecyl Sulphate; CTAB: Cetyl Trimethylammonium Bromide; rpm: Rounds Per Minute; MTCC-Microbial Type Culture Collection; IMTECH: Institute of Microbial Technology

Introduction

The bacterium *Staphylococcus aureus* is a common cause of foodborne illness in humans. It is also commonly known as "Staph aureus". The bacterium produces toxin which causes the disease. *S. aureus* lives in the nasal passages, throat, and on the hair and skin of healthy individuals. However, incidence is higher in those people who are around sick individuals, such as health professionals. *S. aureus* are observed available in air, dust, sewage, water, milk, food, food equipment, environmental surfaces, humans, and animals. Even though food handlers are generally the key source of food contamination in *S. aureus* associated foodborne outbreaks but equipment and environmental surfaces also contribute as important sources of contamination. *Staphylococcal* food poisoning is characterized by sudden onset of nausea, vomiting, stomach cramps and mostly diarrhoea episodes. The disease symptoms generally appear within 30 minutes to 8 hours after consuming contaminated food, and last upto 24 hours [1].

According to an estimate, *Staphylococcal* foodborne intoxications have been contributed to 1,513,000 cases of illnesses and 1,210 deaths annually in the United States with an estimated cost of $6.8 billion. Among all major foods, beef is one of most frequently perishable food due to its excellent nutritive composition which supports the microbial growth. In past, numerous foodborne outbreaks have been documented linked with consumption of *S. aureus* contaminated beef and beef products [2-5]. During beef handling poor hygienic and handling procedures are key source of *S. aureus* contamination and dissemination in beef derived food materials. However, other various factors could also be associated in beef contamination at any point along the food chain, including the environment and animal handling and processing procedures of slaughtering [6]. Therefore it is important to adopt the safe hygienic and handling procedure to produce the safe food for public consumption. Food safety and standard authority of India (FSSAI) and other food quality regulating authorities in other countries established *S. aureus* limit in fresh meat including beef. FSSAI established zero tolerance of *S. aureus* in 25g of food products including meat items [7]. However variation in *S. aureus* limit observed such as International Commission for the Microbiological Specifications of Foods has established maximum allowed *S. aureus* counts as 4 $\log_{10}$ cfu/g [8]. The burden of illness caused by *S. aureus* is growing regularly worldwide even though it is a major concern of public health programs.

Moreover, Antibiotic resistance is a natural phenomenon developed in bacteria to protect them in chemically stressed environment, and episodes of antibiotic resistance development and rise of multidrug resistant (MDR) in *S. aureus*, are posing more complications in management of associated infections of the bacteria. The incidences and rise of drug resistant *S. aureus* are documented worldwide [9-11]. The true incidence of *staphylococcal* food poisoning is underestimated due to improper sample collection, misinterpretation of results diagnosis procedures and under-reporting of true cases. Due to poor hygienic practices and low level of awareness, this problem is worse in developing countries including India. In best of our knowledge, *S. aureus* prevalence and multidrug resistance from various meat sources are documented [12]. However, the systematic studies from beef have neglected. Therefore, present study is demeanoured on contamination and prevalence of *S. aureus* with special emphasis on multidrug resistance from beef.

Materials and Methods

Reference and standard bacterial cultures utilized in this study

The standard bacterial strains used in this study and their sources are listed in table 1.

Name of the Organism	Source
S. aureus (MTCC[a] 1145)	Division of Standardization, IVRI, Bareilly, India
S. epidermidis (MTCC[a] 435)	Division of Veterinary Public Health, IVRI, Bareilly, India
Listeria monocytogenes (MTCC[a] 657)	IMTECH , Chandigarh, India
Rhodococcus equi (MTCC[a] 1135)	Division of Veterinary Public Health, IVRI, Bareilly, India
Bacillus subtilis (MTCC[a] 121)	Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India
Shigella sp. (SM[c] 07)	Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India
Citrobacter sp. (EN[c] 06)	Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India

Table 1: Detailed list of Standard/Reference bacterial strains used in this study.

[a] Microbial type culture collection.

[c] Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India.

Collection and laboratory transfer of food samples

Fifty (50) buffalo beef samples were collected from the local butcheries shops in Bareilly, Northern India according to USFDA/BAM/CFSAN [13]. In concise, 100g of beef sample from buffalo were collected in sterile screw-cap jar containing 100 ml buffered peptone water (0.1%) maintaining aseptical conditions and placed in an ice-box for transferring to the laboratory without exposure to direct sunlight. The conventional cultural methods were commenced on the same day for the identification of *S. aureus*.

Isolation and confirmation of *S. aureus* by conventional cultural practices

S. aureus isolation was processed by following USFDA/BAM/CFSAN procedure [14]. Briefly, 25g beef sample was homogenized using a stomacher in 225 ml of Tryptic soy broth (TSB) broth (Hi-media, India) containing sodium chloride (10%) and sodium pyruvate (1%) for 2 min and these broth samples were then incubated at 35°C for 48h for enrichment. Further, a bacterial inoculum of these enriched broth was streaked on the solid agar medium Baird Parker (BP) agar (Hi-media) containing selective supplement (egg yolk emulsion and 3.5% potassium tellurite) and incubated for 48 h at 35°C. Bacterial colonies displaying typical features of *S. aureus* were segregated by repetitive streaking. The assessment of the morphological features of typical *S. aureus* growth on Baired Parker (BP) agar was made by selecting the colonies grow as circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet-black, frequently with light-coloured (off white) margin, surrounded by opaque zone, frequently with an outer clear zone with buttery to gummy consistency. The microscopic examination after Gram staining conferred the Gram-positive cocci clusters resembles as bunches of grapes. To confirm *S. aureus*, an assembly of biochemical assay including glucose utilization, oxidase, catalase, mannitol, coagulase, growth in 10% NaCl, and DNAse was selected as described previously by USFDA/BAM/CFSAN [14]. These biochemical assays were carried out as narrated previously [15]. At last, a separate specific isolate number was allocated to recognize the colony and maintained in BHI broth with 20% glycerol and stored at -20°C.

Enumeration of *S. aureus*

Enumeration of bacteria was performed by using the standard procedures of International Organisation of Standardization (ISO) [16]. Concisely, 25g beef sample was homogenized in 225 ml of Tryptic soy broth (TSB) broth (Hi-media, India) containing sodium chloride (10%) and sodium pyruvate (1%) for 2 min using a stomacher. These homogenized samples diluted serially for achieving appropriate tenfold dilutions and 0.1 ml aliquot was processed on Baired Parker Agar (BPA) in triplicates for enumerating *S. aureus* using spread-plate procedure.

Confirmation of nuclease virulence factor among *S. aureus* isolates by PCR

The confirmation of virulent *S. aureus* was carried out by detecting *nuclease* (*nuc*) gene among *S. aureus* isolates using PCR. For this DNA extraction kit (Genei, Banglore, India) was used for extracting genomic DNA as described previously [17]. In brief, one ml of overnight Brain Heart Infusion (BHI) broth culture was used from loopful colony of bacterial pathogen followed by centrifugation at 6000 rpm for 15 min at 4^0C. The obtained pellet was washed using 100 µl TRIS-EDTA (TE) buffer twice. Now, this pellet was suspended in 567 µl of TE buffer (10 mM Tris, 1 mM EDTA) by mild vortexing. Next, 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase-K (100µg ml/ml in 0.5% SDS) were added in the above suspension and mixed softly by inversion followed by incubation for one hour in a water bath maintaining 37°C temperature. Then 100 µl of 5M NaCl was added and mixed softly followed by addition of 80 µl of CTAB/NaCl (10% CTAB dissolved in 0.7M NaCl). The resulting suspension was mixed properly and incubated at 35^0C for 10 min again in a water bath. Now, the equal volumes of (0.7-0.8 µl) of phenol; chloroform; isoamyl alcohol (25:24:1) were also added and mixed diligently. The suspension was further centrifuged at 13,000 rpm for 20 min which resulted in formation of a thick protein layer and polysaccharide at the interface of the two phases. The top layer or aqueous phase was smoothly segregated and cautiously transferred to a fresh microcentrifuge tube. After consecutive repeatation twice of this step, the obtained final supernatant was mixed with 0.6 volumes of isopropanol at room temperature and centrifuged at 13000 rpm for 5 min followed by rinsing with 400µl of 70% ethanol. At last, rinsed suspension was centrifuged again at 13,000 for 5 min which resulted in extraction of DNA in pellet. The pellet was air dried carefully and finally resuspended in 50µl of sterile glass distilled water, added RNAase (10 µg ml/ml) and incubated for 20 min at room temperature.

The *S. aureus* isolates were verified for *nuclease* virulence factor targeting *nuc* gene using thermal cycler (Corbett Life Sciences, USA) as recited previously [18] with some adaptations. The primer pairs (F): GCGATTGATGGTGATACGGTT and (R): AGCCAAGCCTTGACGAACTAAAGC were manufactured by Genuine Chemical Corporation, India. The reaction mixture for PCR (25 µl) consisted of 2.5 µl of 10X PCR buffer, 1.0 µl of both forward and reverse primers (15 pmol), 1Uof *Taq* DNA polymerase enzyme and 2.5 µl of bacterial DNA template. The final volume (25 µl) of reaction mixture was maintained by adding nuclease free water. The PCR cycling conditions were optimized as initial denaturation step at 95°C for 3 min followed by 30 subsequent cycles of heat denaturation of 95°C for 30 sec, annealing at 55°C for 75 sec, and extension at 72°C for 75 sec. A final extension was performed at 72°C for 10 min to complete the synthesis of all strands.

The specificity of the assay was maintained using reference/ standard culture of *S. aureus* (MTCC 1145) as positive control whereas *S. epidermidis* (MTCC 435), *L. monocytogenes* (MTCC 657), and *Rhodococcus equi* (MTCC 1135), *B. subtilis* (MTCC 121), *Shigella sp.*(SM-07) and *Citrobacter sp.* (EN-06) cultures as negative controls. PCR products were separated through agarose gel electrophoresis (1.5%), stained with ethidium bromide (Sigma-Aldrich, USA), and visualized under ultra violet light.

Determination of antibiotic resistance

All the *S. aureus* isolates were scrutinized for antibiotic resistance examined by disc diffusion method as recited previously [19]. A total seven commercial sensitivity discs (Hi Media) that belong to five groups of drugs viz. Penicillin (10 units) and Ampicillin (10 µg) (Penicillins or beta lactam), Chloramphenicol (30 µg) (Phenicols), Streptomycin (10 µg) and Kanamycin (30 µg) (Aminoglycosides), Lincomycin (2 µg) (Lincosamide), Erythromycin (15 µg) (Macrolides) were scrutinized. In concise, 0.1 ml of *S. aureus* suspension (10^8 cfu/ml) was prepared in Brain Heart Infusion (BHI) broth (Pronadisa, Spain) and spread onto Muller Hinton (MH) agar (Hi-Media) plates. These plates were incubated at 37°C for 18 h and the sensitivity of antibiotics was examined measuring the inhibition zone diameter of bacterial growth around the antibiotic disc.

Results and Discussion

Enumeration of *S. aureus* in food samples

Staphylococcus aureus is one of most frequently implicated bacterial pathogen in food borne illnesses and meat products including beef may get contaminated with the pathogen due to handling and hygienic practices applied during the slaughtering of animal [20]. The level of *S. aureus* contamination in beef was also found in considerable range of $\log_{10}$1.0 to 3.6 in the present study (Table 2). The close similar contamination of *S. aureus* from beef also has been noticed in other studies from all over the world. The mean *S. aureus* count of $\log_{10}$ 3.40 ± 0.63 in ready-to-eat raw beef samples of Bahir Dar city, Ethiopia reported [21]. In a study conducted in Gharbia governorate of Egypt, the mean *S. aureus* count was observed as $1.87x10^3$ ± $0.36x10^3$ from beef burger [22]. A relatively high count $\log_{10}$4.13 ± 0.0132 to $\log_{10}$ 5.97 ± 0.0599 was evaluated in a study conducted in Bangladesh during the assessment of microbiological quality of raw meat and meat products including beef [23]. In India, the presence of *S. aureus* is not permissible in per 25g of meat [7]. However in our study estimated load of $\log_{10}$1.0 to log 3.6 and most of the samples 77.7% contaminated with the load of $\log_{10}$1.0 to log 2.9 (Figure 1). The contamination of beef with *S. aureus* has been noticed due to poor personnel hygiene and method used for slaughtering of the animal [24].

Sample No.	Source	*S. aureus* count log cfu/g (Mean ± SD)*
B-1	Beef	1.8 ± 0.2
B-4	Beef	3.0 ± 0.4
B-5	Beef	2.5 ± 0.1
B-11	Beef	3.6 ± 0.2
B-12	Beef	2.8 ± 0.1
B-14	Beef	2.6 ± 0.1
B-21	Beef	1.0 ± 0.1
B-24	Beef	1.6 ± 0.2
B-27	Beef	1.6 ± 0.2
B-28	Beef	1.8 ± 0.1
B-29	Beef	1.6 ± 0.3
B-32	Beef	2.8 ± 0.1
B-34	Beef	2.6 ± 0.1
B-35	Beef	3.0 ± 0.2
B-36	Beef	2.0 ± 0.2
B-39	Beef	3.0 ± 0.1
B-41	Beef	2.6 ± 0.2
B-42	Beef	2.8 ± 0.2

Table 2: *S. aureus* count from beef samples.

* Values are log mean ± SD.

SD; Standard deviation.

Figure 1: Analysis of *S. aureus* count (log cfu/g) from different beef samples.

Prevalence of *S. aureus* in beef samples

In this study the prevalence of *S. aureus* was scrutinized in beef samples of buffalo origin collected from local butchery shops in Bareilly city, Northern India. The prevalence of *S. aureus* in beef samples has shown in table 3. *S. aureus* was isolated from 18 (36.0%) of beef samples using conventional cultural methods and confirmed by using *nuc* gene based PCR assay. Hence verified the similar *S. aureus* prevalence 18 (36.0%) in beef samples using PCR assay as observed by cultural methods. The *S. aureus* isolates were confirmed by detecting *nuclease* virulence factor using PCR. An amplified PCR product of molecular weight 270bp was noticed from *S. aureus* MTCC1145 from *nuc* gene based specific PCR reaction (Figure 2). The virulence factor *nuclease* was detected in all 18 (100%, n = 18) *S. aureus* isolates. The specificity of the PCR assay was established using the specific primers targeting *nuc* gene detection for *S. aureus* corroboration (Figure 2). The results indicated the specificity of primer pairs towards *S. aureus* and an unambiguous PCR product after amplification was attained. No non-specific amplification product was scrutinized in PCR assay. A satisfactory literature on expression and secretion of an extracellular *nuclease* by *S. aureus* is available and states that *nuclease* factor contributes an important role in pathogenesis of *S. aureus*. The contribution of *nuclease* expression by *S. aureus* in biofilm dispersal and subsequent promotion of disseminating the organism has been recited also. A number of investigators used *nuclease* factor (*nuc* gene) for the identification of *S. aureus* and found similar results as obtained in this study [25-27].

Type of sample	Total number of samples screened	Number of positive samples by cultural method	Number of positive samples by PCR method	Number of positive samples for *nuc* gene among *S. aureus* isolates by PCR
Beef	50	18 (36.0%)	18 (36.0%)	18 (100%)

Table 3: Prevalence of *S. aureus* and *nuclease* (*nuc*) gene in beef samples.

Figure 2: Specificity of *nuc* gene (270 bp) based PCR reaction for the confirmation of *S. aureus*. (a) Lane M: DNA ladder (100 bp), Lane 1: *S. aureus* (MTCC 1145), Lane 2: *S. epidermidis* (MTCC 435), Lane 3: *L. monocytogenes* (MTCC 657), Lane 4: *R. equi* (MTCC 1135), Lane 5: *Bacillus subtilis* (MTCCa 121), Lane 6: *Shigella sp.* (SM^{c} 07), Lane 7: *Citrobacter sp.* (EN^{c} 06).

In best of our knowledge, *S. aureus* prevalence in different types of meat and milk is available sufficiently from India [28-31]. However it lacks information from beef. In the present study, *S. aureus* prevalence 36.0% in beef was observed which is observed slightly higher than prevalence (28.0%) reported in a study from India recently. On the other side, *S. aureus* prevalence range 3.0-75.0% has chronicled from all over the globe such as Egypt (3-52.0%), South Africa (3.2%), Ethiopia (16.6%), Libya (18.0%), USA (20.5%), Mexico (38.8%), Turkey (63.5%), Pakistan (75.0%) [32-38]. The variation among the contamination of *S. aureus* may be multifactorial. It might be due to disproportion of sample size and isolation approach used, awareness and abilities of the butcher or meat handling personnel, management system, and topographic regions [39-41].

Occurrence of multidrug resistant (MDR) *S. aureus*

The antibacterial resistance profile of eighteen *S. aureus* isolates was scrutinized against seven antibiotics. The antibiotic susceptibility profiles verified drug resistance among 94.4% isolates. The highest resistance 72.2% was observed against penicillin whereas as a comparative lower resistance 44.4% was observed against ampicillin. On the other side, a close range of resistance 22.2-33.3% was observed against erythromycin, lincomycin, streptomycin and kanamycin. Lowest resistance (16.6%) was observed against chloramphenicol (Table 4). The antibiotic resistance among *S. aureus* ranged from 33.3-75.0%, against penicillin, 33.3-50.0% against ampicillin and 23.0-50.0% against erythromycin has been filed from different studies [42-44]. These testimonies suggested chloramphenicol, kanamycin and erythromycin could be drug of choice for the treatment of *S. aureus* associated infections. In the present investigation, 6 (33.3%) isolates were resistant to four to five antibiotics and total forteen resistance patterns were displayed by eighteen isolates of *S. aureus* (Table 5). The most common resistance pattern was witnessed against penicillin and ampicillin and witnessed among 15 (83.3%) *S. aureus* isolates. Likewise in a study from India, the resistance pattern confirmed the penicillin and ampicillin associated resistance among most of the *S. aureus* [45].

Name of antibiotic	Antibiotic class	Number of resistant isolates (%)
Penicillin (P)	β-lactams	13 (72.2)
Ampicillin (Am)	β-lactams	8 (44.4)
Chloramphenicol (Cl)	Phenicols	3 (16.6)
Streptomycin (S)	Aminoglycosides	5 (27.7)
Kanamycin (K)	Aminoglycosides	6 (33.3)
Erythromycin (E)	Macrolides	4 (22.2)
Lincomycin (L)	Lincosamides	5 (27.7)

Table 4: Prevalence of antibiotic resistance of *S. aureus* isolates.

Isolate No.	Antibiotic resistance profile	Number of Isolates
BSA – 27, BSA – 28, BSA – 29	P	3
BSA – 21	S	1
BSA– 14	Am	1
BSA – 11	Am, P	1
BSA – 5	K, L	1
BSA – 32	Am, L	1
BSA – 12	P, S	1
BSA – 4	Am, P, S	1
BSA – 1	L, P, S	1
BSA – 41, BSA - 42	Cl, E, K, P	2
BSA – 36, BSA - 39	Am, E, K, P	2
BSA – 34	Am, Cl, L, P	1
BSA - 35	Am, K, L, P, S	1
BSA - 24	-$^{\Psi}$	1

Table 5: Antibiotic resistance pattern of *S. aureus* isolates (n* = 18).

n* Total number of isolates screened.

-$^{\Psi}$ either sensitive/intermediate to antibiotics used.

Am- Ampicillin, Cl- Chloramphenicol, E- Erythromycin, K- Kanamycin, L- Lincomycin, P- Penicillin, S- Streptomycin.

Multidrug resistance among *S. aureus* has been chronicled in various studies from all over the world. In Colombia, South America low (2.0-12.0.3%) MDR was monitored among *S. aureus* from different meat and beef samples [46]. In Turkey 96.8% MDR *S. aureus* were filed from meat samples collected from local retail supermarkets at Ankara [37]. In South Africa, relatively high 15.3% MDR than the Colombia, South America among *S. aureus* was filed from beef and beef products [38]. Similar close 92.0% MDR *S. aureus* were reported from Northwest and Southwest regions of Cameroon [47]. The inconsistency of MDR among *S.*

aureus from various reported information and our study may due to the different criteria used for concluding of MDR and geographic location. In above mentioned studies, resistance against three or more antibiotics considered as MDR (≥ 3) whereas in the present study the criterion for MDR was approved as defined by CDC [48]. It states resistance to one antibiotic in three or more antibiotic category or class has to be considered as MDR. Therefore, MDR (≥ 1) was recorded in 94.4% *S. aureus* isolates (Figure 3). A comparison of MDR *S. aureus* has depicted from neighbouring countries and different parts of the world has shown in figure 4 [21,49-58].

Figure 3: Analysis of Multidrug Resistance (MDR) among *S. aureus* isolates.

Figure 4. Prevalence of Multidrug Resistance (MDR) among *S. aureus* from different countries (*Data not available).

In past two decades, the extensive use of antibiotics in bacteria is raising the emergence of multidrug resistant strains (MDR), which exhibited great challenges to public health. As a terrible adaptive capacity of *S. aureus*, bacteria can adapt different environmental situations and quickly become resistant to almost all antibiotics. In these days, more and more MDR *S. aureus* are reported in foodborne illness and poisoning outbreaks. In conclusion, best of our knowledge, the prevalence of antibiotic resistant *S. aureus* in beef of buffalo origin presented in this study was first time reported from northern India and observed alarmingly higher. Multidrug resistance was also observed with high incidence. The prevalence of resistance among food borne bacterial pathogens has increased due to increase in number of food borne infections and the corresponding use of antimicrobials also. Moreover, increase of food requirements for increasing population has led to the widespread routine use of antimicrobials as growth promoters or preventive agents in food producing animals and poultry flocks. Global concern over the misuse of antimicrobials and subsequent emergence of antibiotic resistant microbe has increased during last two decades. Constant and irrational use of antibiotics in both veterinary and human medicine, especially in developing countries coupled with current knowledge of transfer of antibiotic resistance among various bacteria makes it essential to monitor the susceptibilities of known pathogenic bacteria to antibiotics [59]. Further, the environmental, eco-social status overcrowding, close association between human and animals and poor hygienic conditions in the area of study might have significantly contributed role in microbial contamination of food chain in developing countries like India. The transmission of theses human pathogens in food chain and indiscriminate use of antibiotics might have resulted in increased emergence of resistance to common antibiotics used in veterinary and medical [60].

Conclusion and Recommendations

In this study the beef samples screened were found contaminated with multidrug resistant *S. aureus* which can be a vital threat to health of consumers in future if the present beef handling practices are continue. Therefore the hygienic conditions in relation to beef safety must be maintained and improved in the study area to avoid the *S. aureus* infections. These infections may be treated with chloramphenicol and erythromycin. However it is important to trace out the point of contamination during the beef production in these butcheries so necessary actions can be applied in such butcheries. At the same time educate the meat handlers for safe handling procedure such as HACCP and Good manufacturing Practices (GMPs) intended to maintain the beef quality. It is also required to educate buffalo farmers, veterinarians, and consumers on the threat of antibiotic resistance. This awareness among

farmers, veterinarians and the consumers can play a crucial role in reducing antibiotic use in animals and therefore the emergence of such MDR pathogens can be regulated.

Declaration of Competing Interest

None.

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Author Contribution

Javed Ahamad Khan carried out experimental work and organized a rough sketch of the manuscript with Fohad Mabood Husain. Rubina Gill ensured grammatical errors. Ram Swaroop Rathore supervised the experiment designing. Iqbal Ahmad supervised and edited the concluding draft of the manuscript. Javeed Akhtar carried out co-editing in reference preparation.

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