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Research Article
Methicillin Resistant *Staphylococcus aureus* Isolated from Wounds of Livestock and Companion Animals of Uttar Pradesh India: A Preliminary Study

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Abstract

Background: *Staphylococcus aureus* is the most common bacterial pathogen responsible for acute as well as many chronic infections in humans and animals as well. The emergence of antibiotic resistance among these pathogens is an issue of global worry. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) is staphylococci showing multiple drug resistance to a large number of antimicrobials including penicillin, methicillin, β-lactam antibiotics, aminoglycoside antibiotics, macrolide antibiotics, etc. **Objective:** The aim of present study was to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical cases of wounds in animals of different species of various age groups in Mathura and nearby areas of Uttar Pradesh, India. **Materials and Methods:** A total of 194 wound samples from cattle, buffalo, dogs, goats, sheep and horses were collected aseptically between 2012-2014. Samples were subjected to microbiological investigation for laboratory isolation, identification and confirmation of causative agents as per the standard protocols. All *S. aureus* isolate were identified and confirmed on the basis of morphology, cultural characteristics and biochemical tests. **Result:** Out of 194 samples, 9 were negative while 185 wound samples revealed 69 isolates of *Staphylococcus aureus* with the prevalence rate of 37.3%. The results also revealed that the incidences of *Staphylococcus aureus* in wound cases were higher in equines (57.14%), cattle (48.28%) and dogs (48.08%) in comparison to buffaloes (24.32%). All the isolates were subjected to drug sensitivity by disc diffusion method to assess the antibiotic resistance against 23 antimicrobials. Results of the current study revealed maximum sensitivity to gatifloxacin (94.20%) and 100% resistance against kanamycin, colistin, clindamycin, penicillin-G, cotrimoxazole and cefotaxime. **Conclusion:** Out of 69, 66 isolates were found to be methicillin-resistant, while 63 were vancomycin resistant and is a noble finding in case of animals in UP. The higher prevalence of methicillin resistant bacterial strains of *Staphylococcus aureus* bacteria from wound infections was an important finding of the study and clearly indicated increase in drug resistance with alert to look for alternate therapeutic treatment.

Key words: *Staphylococcus aureus*, animals, wounds, MRSA, antibiotic resistance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indiscriminate uses of antibiotics and drugs prescription without proper susceptibility tests for quick medication are some key factors for emergence of resistant organisms against antimicrobial drugs especially in developing nations[1,2]. The emergence of antibiotic resistance among pathogens is a rising issue of global worry[3,4]. Among various pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) is staphylococci showing multiple drug resistance to a large number of antimicrobials including penicillin, methicillin, β-lactam antibiotics, aminoglycoside antibiotics, macrolide antibiotics, etc. Over past several years different studies conducted all around the world have alarmed the presence of MRSA strains of bacteria from hospital, clinics, community, farm and environmental settings[5-8].

The presence of MRSA is reported from human as well as from different species of animals including cattle, horses, sheep, cats, dogs and chickens in different regions of world[9]. The MRSA can be transferred among closely kept animals and from animals to human which reflects that farm workers, veterinarians, persons working at abattoirs are more prone and at highest risk. Due to transfer of MRSA bacteria among domestic pets, farm animals, livestock, human and feral/wild animals probability of emergence of new strains of MRSA also expanded due to genetic recombination[10,11].

The present study was planned to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical cases of wounds in animals of different species of various age groups in Mathura and nearby areas of Uttar Pradesh, India. For this, a total of 194 wound samples from cattle, buffalo, dogs, goats, sheep and horses were collected aseptically by Levine's technique in period of 2 years between 2012-2014. Collected clinical samples were subjected to microbiological investigation for laboratory isolation, identification and confirmation of causative agents as per the standard protocols. All the isolates were subjected to drug sensitivity by disc diffusion method to assess the pattern of antibiotic resistance against 23 antimicrobials.

In the absence of appropriate surveillance of antimicrobial resistance, growing increase in MRSA bacteria appended worries in the incidence of infections among variety of wounds and demands constant bacteriological monitoring of the pathogen even from minor injuries to know their current antibiotic susceptibility pattern[12-15]. Therefore, this study was intended to determine the prevalence of MRSA from infected wounds to support the empirical therapy with precise medication as per the specific cause.

MATERIALS AND METHODS

Study design and wound sample collection: A total of 194 samples of wound were collected from various animal species including cattle, buffalo, goats, sheep, horses and dogs to screen for bacterial causes from clinical cases and field in and around the Mathura city (Table 1). Swab samples from wounds of various types viz., horn abscess, gangrenous wound, chronic abscess, deep suppurative wounds, open, gun-shot, lacerated, abrasive, incised, ulcerated and post-operative wounds (Fig. 1, 2) were collected by Lavine's aseptic technique after cleaning the periphery of wound with 70% ethanol swabs. Samples from deep wounds were collected using sterile cotton-tipped swabs (PW003, HiMedia) by taking all aseptic precautions to check the contamination while sample collection. The swab samples were directly submitted to the Department of Veterinary Microbiology, College of Veterinary Sciences, DUVASU University, Mathura (U.P.), India for further laboratory processing by standard procedures.

Wound sample processing for isolation and identification of bacterial agents: During the study period, a total of 194 infected wound samples were selected and swab samples were processed for isolation and identification of different aerobic and anaerobic bacterial agents based on the initial screening and characteristics according to the standard operating procedures of the laboratory. Upon receipt as per

Table 1: Species-wise distribution of wound samples and isolates percentage of *Staphylococcus aureus*

Species affected	Distribution of wound samples species-wise		Total No. of sample collected	Isolates of *S. aureus*	Within species percentage of *S. aureus* isolates	Species-wise Isolates percentage of *S. aureus*
	Positive	Negative				
Buffalo	74	2	76	18	18/74 = 24.32	18/69 = 26.09
Cattle	29	1	30	14	48.28	20.29
Canine	52	3	55	25	48.08	36.23
Equine	14	2	16	8	57.14	11.59
Sheep	6	-	6	1	16.67	1.45
Goat	8	1	9	3	37.5	4.35
Total No. of samples	185/194 (95.36%)	9/194 (4.64%)	194	69/185 = 37.3%		

Fig. 1: Open deep wound in buffalo

Fig. 2: Lacerated wound in horse

Fig. 3: *Staphylococcus aureus* over NA

Fig. 4: Haemolysis on blood agar

Fig. 5: *Staphylococcus* spp., over MSA

biochemical tests (Fig. 3-5). The generic identification of bacterial isolates was carried out as per the techniques of Cowan and Steel[16] and further speciation was performed as described by Quinn *et al.*[17].

Susceptibility of bacterial isolates to antimicrobial agents:
Bacterial isolates were subjected to antibiotic sensitivity testing against 23 different antimicrobial discs by disc diffusion method according to CLSI[18] to assess the pattern of antibiotic activity (Fig. 6). All the bacteria were subjected to antibiotic sensitivity testing (ABST) against different classes of antimicrobials: Penicillin class (ampicillin (AMP), amoxycillin, ampicillin/sulbactum (A/S), penicillin-G (P-G), methicillin (MET)), fluorquinolones class (ciprofloxacin (CIP), norfloxacin (Nx)), cephalosporin class (cefotaxim (CTX), ceftriaxone (CTR)), aminoglycosides class (streptomycin (S), gentamycin (Gen)), tetracycline class (T), folate pathway Inhibitors (cotrimoxazole (Co-T)), phenicols class (chloramphenicol (C)), glycopeptides class (vancomycin (Va)), macrolides class (erythromycin (E)) and lincosamides class (clindamycin (CD), amikacin (AK),

the history of sample each swab was inoculated into Nutrient Broth (NB) or Robertson Cooked Meat (RCM) media and accordingly incubated at 37°C upto 24-48 h under aerobic or anaerobic conditions, respectively before attempting pure colony isolation over solid nutrient medium. Upon isolation, identification was followed as per cultural, morphological and

Table 2: Result of antibiotic sensitivity testing of various isolates and percentage sensitivity of isolates recovered from wounds of animals

Name of microorganism	Total No. of isolates	Antibiotic discs used (No. of sensitive isolates)																						
		AMP	AMC	A/S	AK	E	GEN	K	MET	NX	P-G	CIP	C	S	T	En	VA	CTR	CTX	Co-T	GAT	SPX	CD	CL
Staphylococcus aureus	69	1	2	6	22	8	13	R	3	10	R	1	25	15	12	4	6	7	R	R	65	11	R	R
Percentage sensitivity	69	1.49	2.98	8.96	32.84	11.94	19.4	0	4.47	14.9	0	1.49	37.31	22.39	17.91	5.97	8.96	10.45	0	0	94.20	16.42	0	0

AMP: Ampicillin, AMC: Amoxycillin, A/S: Ampicillin/sulbactum, AK: Amikacin, E: Erythromycin, CTX: Cefotaxime, GEN: Gentamicin, CIP: Ciprofloxacin, K: Kanamycin, MET: Methicillin, T: Tetracycline, Nx: Norfloxacin, P-G: Penicillin-G, C: Chloramphenicol, S: Streptomycin, En: Enrofloxaacin, Va: Vancomycin, CTR: Ceftriaxone, Co-T: Cotrimoxazole, GAT: Gatifloxacin, SPX: Sparfloxacin, CD: Clindamycin, CL: Colistin, R: Resistance, S: Sensitive

Fig. 6: Disc diffusion method (ABST)

kanamycin (K), enrofloxacin (En), gatifloxacine (GAT) sparfloxacine (SPK) and colistin (CL)) by disc diffusion technique of Kirby-Bauer and results were interpreted as per the standard charts provided from the drug supplier (HiMedia, India)[19].

RESULTS

In present study out of 194, a total of 185 samples revealed presence of different species of bacteria with 69 isolates of *Staphylococcus aureus*. Out of 194, 9 (4.64%) samples of wounds were negative and 185 ((95.36%) were positive. Microbiological investigation based upon cultural, morphological and biochemical studies revealed isolation of strains of *S. aureus, Pseudomonas aeruginosa, E. coli, Klebsiella* spp*., Protius, Bacillus, Clostridium, Streptococcus, Micrococcus* with maximum prevalence of *S. aureus* strains comprising 37.3% of total. The majority of wounds contained a mixture of aerobes and anaerobic bacteria reflecting the diverse micro-biota present in the atmosphere in vicinity of the affected animals. Among animals, recovery of *S. aureus* was maximum from wound samples of canines (36.23%) followed by buffalo (26.09%) and cattle (20.29%). Though within a particular animal species wound samples from

equines revealed maximum occurrence of *S. aureus* (57.14%), followed by cattle (48.28%), canines (48.08%), buffaloes (24.32%), goats (37.5%) and sheep (16.67%). The results of frequency distribution of *S. aureus* from different species were presented in (Table 1).

The result of *in vitro* antibiotic sensitivity testing of all isolates against 23 different antibiotics such as ampicillin, amoxycillin, ampicillin/sulbactum, amikacin, erythromycin, gentamicin, kanamycin, methicillin, norfloxacin, penicillin-G, ciprofloxacin, chloramphenicol, streptomycin, tetracycline, enrofloxacin, vancomycin, ceftriaxone, cefotaxime, cotrimoxazole, gatifloxacine, sparfloxacine, clindamycin and colistin was recorded and compared with the standard charts (Fig. 7). Antibiotic sensitivity pattern of the *S. aureus*, isolated from different cases of skin/wound infections, revealed resistant strains even against multiple drugs. Most of strains were sensitive to gatifloxacin (94.20%). Other drugs were comparatively less effective as depicted from percentage of drug sensitivity in Table 2. About 94.20% strains were sensitive to gatifloxacin followed by chloramphenicol (37.31%), amikacin (32.84%), streptomycin (22.39%), gentamicin (19.4%), tetracycline (17.91%), sparfloxacin (16.42%), norfloxacin (14.9%), erythromycin (11.94%), ceftriaxone (10.45%), ampicillin/sulbactum (8.96%), vancomycin (8.96%), enrofloxacin (5.97%), methicillin (4.47%), amoxycillin (2.98%), ampicillin (1.49%) and ciprofloxacin (1.49%) as evident from Table 2. The isolated bacteria were cent-percent resistant to penicillin-G, cefotaxime, kanamycin, colistin sulphate, clindamycin and cotrimoxazole (Fig. 8). The drug resistance profile of tested *S. aureus* isolates showed that 100% of *S. aureus* strains were resistant to penicillin, 98.51% to ampicillin, 97.02% to amoxicillin, 95.53% to methicillin and 91.04% to vancomycin. No drug showed 100% efficacy against *S. aureus* isolates. These methicillin resistant isolates showed resistance to other drugs also which reflects their multi-drug resistance pattern. This study indicated that many isolates were resistant to three or more classes of antimicrobials.
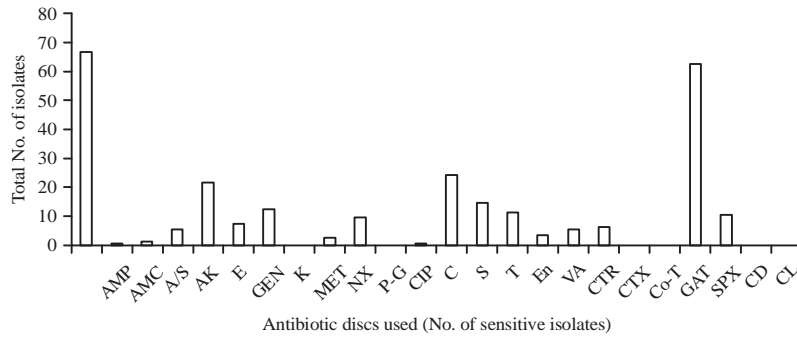
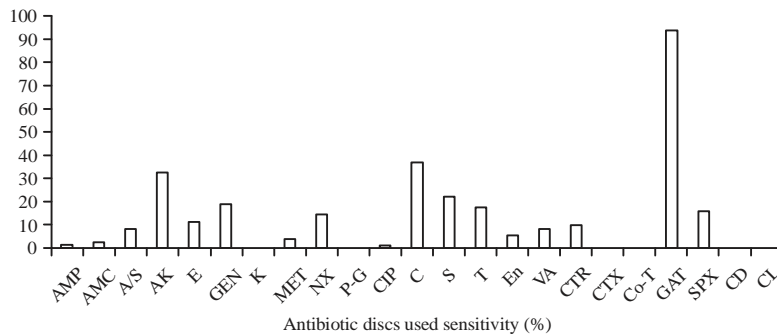Fig. 7: Antibiogram of *Staphylococcus aureus*

Fig. 8: Percentage sensitivity of *Staphylococcus aureus* against various antimicrobial drugs

DISCUSSION

In the study performed over animals to screen the bacterial pathogens of wounds, among significant isolates *Staphylococcus aureus* was the most common isolate (37.3%). This finding is in accordance with the findings of Qureshi *et al.*[20] who reported *Staphylococcus aureus* as maximum isolated bacteria from wounds in camel with the recovery of 40% hemolytic *S. aureus*. These results are also in agreement with other studies depicting 41% *Staphylococcus aureus* isolated from wound infections[21], 60.60% recovery[22] of *S. aureus* from wound cases and 66.7% from another study[23], however percentage of recovery is higher which could be due to change in the study population as higher percentage is reported from hospital cases. *Staphylococcus aureus* is amongst important and major resistant bacterial pathogens to the general population. Bacterial skin infections common in animal veterinary practice are most frequently occurred by *Staphylococcus aureus* as evident from the results. These results are supported by earlier findings of researchers[21], who reported *Staphylococcus* as most common pathogen (23%) isolated from wounds in dogs. The anti-microbial resistance profile of these isolates indicated that methicillin-resistant *Staphylococcus aureus* (MRSA) is now a days frequently involved in animal wound infections and such bacteria are resistant to more than one class of drug which explain the reason behind the chronicity of infection and difficulty in treatment of infections due to multi-drug resistant bacterial strains. These results are in conformity with the reports of Van Duijkeren *et al.*[24] who documented that out of 300 staphylococci isolated from animals 100 strains of *S. aureus* were multi-drug resistant. Work done in developed countries highlighted the emergence and spread of resistance in *Staphylococcus aureus* against penicillin, methicillin, tetracycline and erythromycin groups of drugs with pace[7,25]. This statement is in agreement to the findings of present study where isolated *S. aureus* showed 100% resistant to penicillin, 95.53% to methicillin, 82.09% to tetracycline and 88.06% to erythromycin. But results are in contrast with the study[23] showing only 45.76% isolates to be MRSA out of 236 isolates of *S. aureus.* The emergence of glycopeptide intermediate *Staphylococcus aureus* (GISA) or vancomycin-intermediate *Staphylococcus aureus* (VISA) and resistance against aminoglycoside antibiotics have made the situation more critical[26]. The *S. intermedius*, which is a normal resident in the nasal cavity, oropharynx and the perianal region of animals, can be a transient resident in other sites especially in case of traumatic injury and the organism can be transferred to these

sites from the oral and anal mucosa during grooming practices. Antibiogram study revealed that among the isolated *Staphylococcus aureus* maximum 94.2% isolates were highly sensitive to gatifloxacin while cent-percent resistance was observed against cefotaxime. Results are confirmed by other studies[22] also reporting 81% resistance of *S. aureus* isolates obtained from wound cases to cefotaxime as resistance is towards higher side. Difference in the percentage could be due to geographical variation and frequency of drug usage. Among resistant strains 100% of *S. aureus* strains were resistant to penicillin, 98.51% to ampicillin, 97.02% to amoxicillin, 95.53% to methicillin and 91.04% to vancomycin. Results were supported by findings of other studies which evidenced that *S. aureus* isolates recovered from wounds showed resistance of 100 and 96% to ampicillin, respectively[22,27]. Similar findings were earlier documented by Bozdogan *et al.*[28], Trakulsomboon *et al.*[29] and Falagas and Karveli[30] in their research who had reported the isolation of multi drug resistant bacterial strains from wounds. The result of study conducted by Godebo *et al.*[31] showed that 94.5% of *S. aureus* was resistant to penicillin, 91.8% to ampicillin and 76.7% to oxacillin. The pattern of their result supports the finding of present study and slightly lower percentage could be due to difference in the population under study. The extensive antimicrobial susceptibility to gatifloxacin in the current study may be due to their infrequent use in veterinary practice. In current study 91.04% isolates were resistant to vancomycin and this is in contrast with the study performed earlier at Iran[32] and Nepal[33] which reported resistance percentage of 40 and 21% against vancomycin. It shows that with the continuous use of vancomycin with the time span more resistant strains are evolving globally.

This study also revealed that among animals, recovery of *S. aureus* was maximum from wound samples of canines (36.23%) followed by buffalo (26.09%) and cattle (20.29%). Though within a particular animal species wound samples from equines revealed maximum occurrence of *S. aureus* (57.14%), followed by cattle (48.28%), canines (48.08%), buffaloes (24.32%), goats (37.5%) and sheep (16.67%). These results are supported by another study[34] where recovery of MRSA from canines was 56%.

Several researchers have stated numerous reports/cases of oxacillin/methicillin resistant *S. aureus* from cows in different countries including India, Brazil, Italy, Pakistan, Nigeria, Turkey and the USA[35]. These findings are in almost confirmity with result of another study in Ethiopia[36] where *S. aureus* showed 100% resistant to oxacillin/methicillin (MRSA). However, in present study resistance percentage against methicillin is higher as compared to other reports obtained from Iraq (88%)[22], Ethopia (83%)[37], Nepal (60.6%)[33] and 57.7% from Tamilnadu, India[23]. Documents detailed the recovery of MRSA from equines also from USA, UK, Canada, Austria, Japan and Ireland[38]. O'Mahony *et al.*[34] also supported the finding of present study in which MRSA was isolated from 25 animals with recovery of 56% from canines and 32% from equines and MRSA isolates were resistant to macrolides, lincosamines, tetracyclines and/or fluoroquinolones groups of antimicrobials and are in conformity with our findings.

Comparatively higher recovery of MRSA from dogs could be due to close contact of human with pet animals. Earlier documents also suggested that colonisation of hospital strain of MRSA is increasingly recorded from pets, including cats and dogs, which is an indicative of close contact of human and dogs[39]. In this way pets are acting as reservoir of resistant MRSA strains for re-infecting in-contact human population. This is supported by literature[40] which mentioned the homology of MRSA isolated from animals with human MRSA through PFGE. The MRSA of animal origin is capable of producing the human infections. Through Random Amplification Polymorphic DNA (RAPD) technique it was confirmed that[41] RAPD pattern of bovine MRSA was alike the human MRSA pattern and human/consumers may get the infection of livestock associated MRSA bacteria by contact with infected cattle and consumption of contaminated animal food products[35,42]. Molecular approaches showed that all the methicillin resistant *S. aureus* may not always be "True MRSA" because sometimes horizontal gene transfer helps in genetic exchange of resistant gene among bacteria if they are shared in between different sources. It is documented that farm-animal MRSA and community-acquired MRSA are genetically more identical as compared to hospital-acquired MRSA[38,43].

Not only with direct contact but literature evidenced that MRSA is seeded in manure of MRSA-positive farms and can be a source of environmental spread of this resistant pathogen and helps in ecological emergence of new multi drug resistant strains[44,45]. As vancomycin-resistant MRSA has been emerged due to transfer of vancomycin resistant gene to MRSA. Earlier in European countries some feed additives for farm animals were extensively used which had similarity with vancomycin and drug vancomycin was used for treatment of MRSA. Hence, because of survival pressure MRSA bacteria received vancomycin resistant gene and vancomycin-resistant MRSA emerged[32,46]. Use of certain antibiotics to which MRSA is already resistant, helps in emergence of new multi-drug resistant strains and their eradication would be very difficult.

CONCLUSION

In veterinary practice, wound infections are the most frequent complications which are many times treated without proper anti-microbial tests. The results of the current study projected the growing prevalence of MRSA, VRSA and multi-drug resistant pathogenic strains to support the monitoring and veterinary assistance for specific therapy implementation. This study also recommended that regular testing and survey for detection of resistant *S. aureus* should be critically followed to have a data base regarding drug resistance profile of emerging new strains of bacterial pathogens. It is imperative that government and animal owners both should follow a series of effective measures for preventing spread of MRSA to livestock with specific use of antibiotics after thorough testing.

SIGNIFICANT STATEMENTS

In the present study out of 194, a total of 185 samples revealed presence of different species of bacteria with 69 isolates of *Staphylococcus aureus*. Out of 69, 66 isolates were found to be methicillin-resistant which is a noble finding in case of animals in UP. The higher prevalence of methicillin resistant bacterial strains of *Staphylococcus aureus* bacteria from wound infections was an important finding of the study and as MRSA can be transferred among closely kept animals and from animals to human it reflects that farm workers, veterinarians, persons working at abattoirs are more prone and at highest risk. Study projects the need of new pharmaceuticals to be developed and validated to combat the MRSA infections in animals and humans.

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