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Isolation, Characterization and Antibiotics Sensitivity Pattern of *Staphylococcus aureus* from Man, Animal and Environment

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Abstract: Staphylococcus aureus, an opportunistic pathogen, is a gram positive, spherical or ovoid bacterium that causes serious diseases in men and animals. The occurrence and etiological significance of Staphylococcus aureus was studied in 623 samples which included 157 samples from man, 394 from animals (including mastitic cattle and dogs) and 72 samples from environment (39 from mangers and 33 from milking equipments). Each specimen was obtained aseptically and was subjected to direct smear by Gram's technique as well as cultured on four microbial media (nutrient agar, mannitol salt agar, mlood agar and Staphylococcus agar). The inoculated media were incubated at 37°C for 24-48 hours for microbial growth. Among 623 samples, 35 isolates of S. aureus were obtained, giving prevalence rate of 5.62 per cent. The prevalence of S. aureus in human, animal and environment was 2.55 (4/157), 6.35 (25/394) and 8.33 (6/72) per cent, respectively. Of the 35 isolates of S. aureus, the prevalence noted in human beings, dogs and cows was 13.79, 20.69 and 65.52 per cent, respectively. Similarly, the location-wise prevalence of S. aureus was found to be 2.85 per cent in skin infections, 5.71 per cent in throat swabs, 2.85 per cent in normal human skin, 17.14 per cent in canine otitis, 31.43 per cent in mastitic milk, 8.57 per cent in normal cow milk, 14.28 per cent in udder & teat skin, and 17.14 per cent in milking equipments. All 35 Staphylococcus aureus isolates were subjected to in vitro drug sensitivity testing using seven drugs by disc diffusion technique. Tetracycline was found to be the most effective chemotherapeutic agent (85.72%) followed by enrofloxacin, gentamicin, ampicillin (each 71.43 %), ciprofloxacin, co-trimaxazole (48.57 %) and furazolidone (40.00 %) against S. aureus obtained from different sources. In conclusion, these findings suggest that Staph auerus plays an important role in the pathogensis of mastitis in cows, otitis in dogs and skin infection in human beings, and that tetracycline is the drug of choice followed by enrofloxacin, gentamycicin and ampicillin in the medical and veterinary management of staphylococcosis of these natures.

Keywords: Staph aureus, Men-Animal-Environment, Isolation, Characterization, Sensitivity pattern

INTRODUCTION

Staphylococcosis, an infectious bacterial zoonosis of global significance, is caused by *Staphylococcus aureus* a Gram positive, non-capsulated, non-motile, catalase positive non-sporulated organism, 0.8-1.0 µm in diameter. *Staphylococcus aureus* occurs as normal flora of the skin, nose and mucous membrane of men and animals. The pathogen produces various types of heat-stable enterotoxins and enzymes. It is an important cause of clinical and subclinical mastitis in dairy animals [29] and otitis externa of dogs [35]. The mastitis is of great risk to human health as the mastitogens of public health significance are excreted in the mastitic milk of dairy animals [25]. The organism is also implicated in cutaneous lesions, inflammatory conditions of bones, joints, vascular structure, in septicemia, toxic shock syndrome, pneumonia and meningitis, besides food poisoning [28]. *Staphylococcus aureus* is the chief pathogen responsible for skin and nosocomial infections in hospitals [21]. It can be distinguished from other species of *Staphylococcus* by a coagulase positive test [33]. Most important reservoir of *S. aureus* in cows is the infected mammary gland [14, 32]. The *in vivo* and *in vitro* sensitivity of this organism to different antimicrobials used in treatment of various ailments vary greatly. Hence, the present study was targeted to isolate and characterize *S. aureus* from men, animals and environment of certain ecological areas like dairy farms, primary health centers (PHCs) and veterinary hospitals in Gujarat; and test their *in vitro* antibiotics sensitivity pattern.

MATERIALS AND METHODS

This work was carried out in the Department of Veterinary Public Health of the College at Anand. The locations of sampling included dairy farms, primary health centers (PHCs) and veterinary hospitals in and around Anand city in Gujarat. The sterile cotton swabs of Hi-Media were used in duplicate to collect

clinical samples from the otitis in dogs, the ear, throat, pus forming lesions, nostrils, skin, nail of human and teat orifice, teat and udder of animals as well s cattle manger and milking equipments etc. Milk samples from healthy and diseased/mastitic cows of university farm were collected aseptically in sterile wide mouth glass stoppered bottles after properly cleaning the udder and teats with potassium permanganate solution (1:1000). In all, 623 samples of men, animals and environmental niche were collected aseptically, cultured and were examined for presence of *S. aureus* isolates.

Cultural Examination of Specimens:

The swabs collected from different lesions of cutaneous surface of men, animals and environment as well as heavy loopful of the milk samples were inoculated onto the plates of four different microbial media (nutrient agar, mannitol salt agar, blood agar and Staphylococcus agar) and incubated at 37°C in an incubator. The plates were examined after 24-48 hrs of incubation for bacterial growth and colony characteristics.

Identification of bacterial isolates:

The detailed identification of the isolates was done according to the method described by Malik [16]. The colonies, which appeared smooth pasty and golden yellow in colour on nutrient agar, and revealed grapes like clusters of cocci were tentatively considered as *S. aureus* colonies. Such colonies were subjected to subculturing on various microbial media slants for fermentation tests, Gram's staining and were confirmed by coagulase and catalase tests [16].

RESULTS

In vitro Antibiotics Sensitivity Tests:

All the bacterial isolates of S. aureus (n=35) obtained from different sources such as skin & throat lesions of human, otitis of dogs, and mastitis, normal milk and milking equipments of dairy cows were subjected to standard in vitro antimicrobials susceptibility testing by disc diffusion technique [2]. Antibiotic discs (Hi-Media) of Tetracycline (30 µg), Ciprofloxacin (5 µg), Co-trimaxazole (25 µg), Ampicillin (10 µg), Enrofloxacin (10 µg), Furazolidone (50 µg) and Gentamicin (10 µg) were used for the purpose.

Prevalence of *S. aureus* in Humans:

Out of ten patients with cutaneous lesions attending the OPD of a Primary Health Centre (PHC), *Staphylococcus aureus* was isolated in pure form on microbial media from only one patient (10.00 %, Table 1, Fig. 1). Out of remaining 9 specimens 5 revealed bacteria other than *S. aureus* and 4 were found sterile. For normal skin swabs examined from 37 persons of a livestock farm and PHCs, *S. aureus* was recovered only from one solitary case (2.70 %). A total of 23 persons working either in livestock farm or attending the OPD of PHCs were investigated for the prevalence of *S. aureus* in their throat. *S. aureus* was cultured only from 2 out of 23 swabs (8.70 %) as a pure growth, while six specimens of throat swabs yielded bacteria other than *S. aureus*. Further, none of the 87 swabs cultured from different other body sites of human beings yielded *S. aureus* colonies (Table 1).

Prevalence of *S. aureus* in Animals

Out of the 29 clinical mastitic milk samples collected aseptically from the affected cows and examined culturally, *S. aureus* (Fig. 2) was isolated from 11 (37.93 %) cases, while amongst 78 normal milk samples of apparently healthy cows, *S. aureus* was isolated only in 4 (3.85%) cases (Table 1). Moreover, 51.72 (15/29) and 35.90 (28/78) per cent mastitic and normal milk samples were found sterile on cultural examination and rests showed a mixed isolates other than *S. aureus.*

Out of 28 and 32 swabs cultured from the teat skin and teat orifices of the heifers maintained on the livestock farm, only 3.57 (1/28) and 0.00 (0/32) per cent swabs, respectively, yielded *S. aureus*. Similarly, the swabs were collected from the various body sites, such as the forehead, belly, nose, ear and vagina of bovine heifers to study the ecology of *S. aureus*. No isolations could be made from any of these sites of the heifers.

Out of 32 and 41 swabs cultured from washed (PP lotion) and unwashed udders of the lactating cow, *S. aureus* was isolated from 2 (6.25 %) and 3 (7.31 %) specimens, respectively.

Otitis externa was diagnosed in 33 dogs attending OPD of Veterinary College Clinic, Anand. On cultural examination *S. aureus* (Table 1, Fig. 3) was identified in 6 (18.18 %) dogs of either sex and of different age groups.

Prevalence of *S. aureus* in Environment

From the 33 swabs of milking equipments of a dairy farm, *S. aureus* was isolated in 6 (18.18 %) equipments on microbial media. Further, a total of 39 swabs from cattle manger (calf pen - 21, milking parlour - 18) were collected for the study. Interestingly, none of the specimens yielded growth of *S. aureus* (Table 1, 2).

Overall, species wise prevalence of *S. aureus* was found to be 2.55, 1.82 and 5.26 per cent among 157, 33 and 361 samples examined of men, dogs and cows, respectively, being higher in cows. Moreover, out of 72 environmental samples (dairy equipments and mangers) 8.33 per cent were found positive for *S. aureus* isolates (Table 2).

Table-1: Prevalence of *Staphylococcus aureus* in men, animals and environment

Sr. No.	Type of infection/ Sources	No. of samples examined	Positive for *S. aureus*		% distribution of *S. aureus*
			No.	%	
1	Infected skin (human)	10	1	10.00	2.85
2	Throat (human)	23	2	8.70	5.71
3	Normal skin (human)	37	1	2.70	2.85
4	Otitis (dogs)	33	6	18.18	17.14
5	Mastitis (cows)	29	11	37.93	31.43
6	Normal milk (cows)	78	3	3.85	8.57
7	Different sites of human body (skin, nose, nail, ear)	87	0	0.00	0.00
8	Different sites of animal body (forehead, udder & teat skin, teat orifice, belly, vagina etc)	254	5	1.96	14.28
9	Milking equipments	33	6	18.18	17.14
10	Cattle manger (calf pen, milking parlour)	39	0	0.00	0.00
Total		623	35	5.62	100.00

Table-2: Species wise distribution of *Staphylococcus aureus* strains

Sr. No.	Species	No. of samples examined	Positive for *S. aureus*		% Distribution of *S. aureus*
			No.	%	
1	Human	157	4	2.55	13.79
2	Dog	33	6	1.82	20.69
3	Cow	361	19	5.26	65.52
Total		**551**	**29**	**5.26**	**100.00**
Environment		72	6	8.33	--

Identification of Isolates

All the 35 isolates recovered from total 623 samples of men, animals and environment were identified by studying their gross, cultural, morphological, physiological and biochemical characteristics as per Malik [16]. On nutrient agar culture media all the isolates except of canine origin produced golden yellow colonies after 48 hours of incubation at 37°C (Fig. 1, 2). The isolates of dogs produced small white colonies onto the plates of nutrient media at 37°C (Fig. 3). The smears prepared from these isolates when observed under light microscope after Gram's staining revealed grapes like cocci or irregular clusters (Fig. 4). Sub-culturing of golden yellow colonies on different media slants resulted in fermentation of glucose, lactose, mannitol and maltose; and showed positive results with coagulase and catalase tests.

In vitro Antibiotics Sensitivity Tests

The *in vitro* antibiotics sensitivity testing of the 35 isolates of *S. aureus* from different sources showed the resistance pattern ranging from 14.29 to 60.00 per cent. Tetracycline was found to be the most effective drug (85.72 %) followed by enrofloxacin (71.43 %), ampicillin (71.43 %), gentamicin (71.43 %), ciprofloxacin (48.57 %), co-trimaxazole (48.43 %) and furazolidone (40.00 %). The isolates were highly sensitive to the above antibiotics to the extent of 54.29, 22.86, 14.29, 17.14, 8.57, 5.71 and 0.00 %, respectively (Table 3).

Table-3: In vitro antimicrobial drug susceptibility pattern of S. aureus strain

Sr. No.	Antimicrobial drugs tested	Number of *S. aureus* strains		
		Highly sensitive	Sensitive	Resistant
1	Tetracycline	54.29	31.43	14.29
2	Gentamicin	17.14	54.29	28.57
3	Enrofloxacin	22.86	48.57	28.57
4	Ciprofloxacin	8.57	40.00	51.43
5	Co-trimaxazole	5.71	42.86	51.43
6	Furazolidone	0.00	40.00	60.00
7	Ampicillin	14.29	57.14	28.57

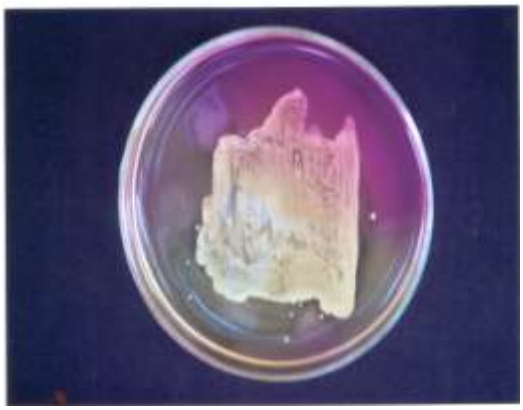

Fig-1: *Staphylococcus aureus* cultured in pure and luxuriant growth from the cutaneous lesions of a 10-year- old girl on Mannitol Salt Agar at 37° C.

Fig-2: Forty-eight hr old growth of *S. aureus* on nutrient agar at 37°C, isolated from milk of a 8-year-old mastitic cow.

Fig-3: Forty-eight hr old growth of *S. aureus* on nutrient agar at 37°C, isolated from a 5-year-old male dog with otitis

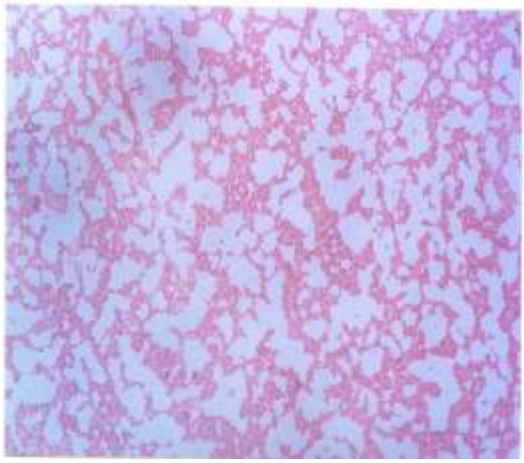

Fig-4: Microphotograph showing grapes like clusters of *S. aureus* in the smear prepared from mastitic milk of a 12-year-old cow. Gram's stain (x 240).

DISCUSSION

Mastitis is the reaction of the milk secreting tissue to injury produced by physical force, chemicals introduced into the gland or most commonly from bacteria and their toxins [4]. However, mastitis is also caused by fungi, actinomycetes and algae [26]. The disease is of great economic importance as it results into tremendous losses to the dairy industry. In addition, it causes great risk to human health as the mastitogens of public health significance are excreted in the mastitic milk of dairy animals [14, 25]. The most important reservoir of *S. aureus* in cows is the infected mammary gland [34]. *Staphylococcus aureus* is a major food borne pathogen due to its capability to produce a wide range of heat-stable enterotoxins. *S. aureus* from infected udders may contaminate bulk milk and, subsequently, raw milk products. Peles *et al.* [27] reported that 14 of 20 Hungarian dairy farms were contaminated with *S. auerus*. 16 (27.1%) of the *S. aureus* isolates tested by multiplex PCR were found to be positive for enterotoxin genes.

Staphylococcus aureus, the principal etiological agent of staphylococcosis, occurs as normal flora of the skin, nose, throat and mucous membrane of man and a wide variety of animals including the avians [24]. The disease staphylococcosis is a highly infectious bacterial zoonosis of global significance, which causes considerable morbidity and mortality among the susceptible subjects [24]. About 15-40 % of healthy humans are carriers of *S. aureus* as they have the bacteria on their skin usually the nostrils without any active infection of disease [5, 18]. *Staphylococcus aureus* (CPS) are considered to be the main bacterial agent in canine otitis [7, 35].

Isolation of *Staphylococcus* from clinical specimens is usually done by inoculating the specimens on nutrient media, staphylococcus agar, mannitol salt agar and blood agar. *Staphylococcus aureus* forms a

fairly large yellow colony on rich medium, *Staphylococcus aureus* is often haemolytic on blood agar but *S. epidermidis* is non haemolytic. *Staphylococci* are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. *Staphylococcus aureus* isolated from the dog produces white colony.

In the present study, the bacterial colonies on nutrient agar plates were usually round, 1.2 mm in diameter, convex, opaque and glistening with an entire edge and soft or butter like in consistency. Typical growth was golden yellow after 24 - 48 hrs of incubation. On blood agar plates, the colonies were usually larger and certain varieties were surrounded by zones of haemolysis. On mannitol salt agar plates, the colonies were usually golden yellow with yellow zones due to fermentation of mannitol. These observations were in accordance with the earlier reports [16, 23].

The present work showed 37.93 per cent incidence of clinical mastitis due to *S. aureus*. The pathogen was also isolated from 3.85 per cent of apparently normal milk samples indicating subclinical mastitis. All the 14 milk isolates of *S. aureus* showed smooth, pasty and golden yellow colour on nutrient agar, revealed gram positive, grape like clusters (cocci) in Gram stain, fermented glucose, lactose, mannitol and maltose; and showed positive results with coagulase and catalase tests. These results simulate with the previous works of isolating *Staphylococcus* spp. from 15 of the 48 quarters showing chronic mastitis (3], and also recovering 46 isolates of *S. aureus* from clinical and subclinical udder infections in 28 Holstein cows [20]. In Orissa state of India, the prevalence of *S. aureus* was found in 33.1 per cent of the subclinically infected quarters [19], whereas in another study *S. aureus* was reported as the chief pathogen (27%) causing mastitis in bovine [6].

The present findings on dairy animals and equipments corroborated well with Lee *et al.* [14], who evaluated the occurrence of *S. aureus* in milk and in the milking environment of 10 small-scale farms (<400 L/d) in Brazil, and isolated *S. auerus* strains from 56 of 849 samples analyzed (6.6%). These included 12 (5.5%) from 220 milk samples of individual cows, 26 (21.7%) from 120 samples of bulk tank milk, 14 (3.6%) from 389 samples from equipment and utensils (teat cups, buckets, and sieves), and 4 (3.3%) from 120 samples from milkers' hands. In our study, *S. aureus* could be cultured from 18.18 % milking equipments as against 100 % reported by Ruzickova [32] from milking machines.

According to Ruzickova [32], environmental sanitation plays pivotal and significant role in the health of animals. We could demonstrate *S. aureus* only in 5 plates (6.94 %) out of 72 plates examined from different areas of the dairy farm (39 mangers and 33 milking equipments). *S. aureus* has also been recovered from air inside a milking parlour [17]. Roberson *et al.* [29] observed 6.85 per cent prevalence of *S. aureus* on the equipment, instrument, housing, water and feedstuffs. In one more study, they [30] could culture the organism from milk (70%, 43 of 61 isolates), heifer body sites (39%, 24 of 61), environmental sites (28%, 17 of 61), or unidentified source (16%, 10 of 61).

In present study, *S. aureus* could not be demonstrated in ear swabs collected from 32 men. This finding is in contrast with Roland and Stroman [31] who isolated 7.8 per cent *S. aureus* from 2048 ears. Similarly, Suzuki *et al*. [36] reported that *S. aureus* is most common bacteria recovered from middle ear discharge of human beings. Otitis externa (inflammation of auditory meatus) is considered the most common disease of the ear canal in the dog sometimes involving the pinna [1]. Recent studies [15, 35] confirmed the occurrence of staphylococcal strains in otitis externa of dogs based on isolation of 44 and 57 strains of *Staphylococcus* from ear discharge of 65 and 96 dogs, respectively, and the coagulase-negative species were the most prevalent, representing 61·3 and 72.0 per cent of the isolates. However, other studies usually reported coagulase-positive species, such as *Staph. intermedius* and *Staph. aureus* as the most frequent Staphylococcus isolates in otitis externa of dogs [1, 7, 8, 12].

The present findings on antimicrobial sensitivity testing of 35 *S. auerus* isolates from different sources indicated that the drug of choice against *S. aureus* induced mastitis and otitis can be tetracycline. Moreover, we noticed resistance pattern of *S. aureus* as 14.29 % to tetracycline, 28.57 % to gentamicin and 51.43 % to co-trimoxazole as against 56.6, 50.7 and 10.2 %, respectively, which concurred with the earlier report [11]. Gentilini [10] recorded that 3.4 % isolates were resistant to gentamicin. Our antibiogram findings also corroborated with several previous studies on bovine mastitic milk [9, 13, 22]. Silva [35] observed CPS strains (28 %; *S. aureus* 8.8%) from otitis externa of dogs to be highly susceptible to enrofloxacin, gentamicin, cephalothin, chloramphenicol and neomycin. Similar results were also noted by earlier researchers [7, 18]. The findings of the present study suggest that tetracycline is the drug of choice in the medical and veterinary management of staphylococcosis.

CONCLUSION

In conclusion, these findings suggest that *Staph auerus* plays an important role in the pathogensis of mastitis in cows, otitis in dogs and skin infection in human beings, and that tetracycline is the drug of choice followed by enrofloxacin, gentamycicin and ampicillin in the medical and veterinary management of staphylococcosis of these natures.

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REFERENCES

1. Acosta B, Real F, Ferrer O et al.; Otitis recidivantes caninas: evaluación del control laboratorial. Med. Vet., 1992; 9: 369-374.
2. Bauer A, Kirby W, Sherris J and Turck M; Antibiotic susceptibility testing. Am. J. Clin. Pathol., 1966; 45: 493.
3. Bhowmik M, Singh S and Iyer P; Studies on chronic mastitis in buffaloes. Indian J. Anim. Hlth., 1977; 16: 157-160.
4. Blood DC and Radostitis OM; Mastitis. In: Veterinary Medicine, 1989; 7th ed. Publ. by Bailliere Tindall Co, London, pp.554-559.
5. Blumberg H; Staphylococcal infections. In: Medicine for Practicing Physician,1996; 4th ed., Appleton and Lange Stamford, Connecticut, pp. 402-406.
6. Char N, Rao P and Rao PVK; Studies on mastitis in buffaloes. Livestock Advisor, 1983; 8: 19-22.
7. Cole LK, Kwochka KW, Kowalski JJ, Hillier A; Microbial flora and antimicrobial susceptibility patterns of isolated pathogens from the horizontal ear canal and middle ear in dogs with otitis media. J. Am. Vet. Med. Assoc., 1998; 212: 534 538.
8. Cox HU, Hoskins JD, Newman SS et al.; Temporal study of staphylococcal species on healthy dogs. Am. J. Vet. Res., 1988; 49: 747-751.
9. Edward M, Anna K, Michal K, Henryka L and Krystyna K; Antimicrobial susceptibility of staphylococci isolated from mastitic cows. Bull. Vet. Inst. Pulawy, 2002; 289-294.
10. Gentilini E, Denamiel G, Llorente P, Godaly S, Rebuelto M and DeGregorio O; Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. J. Dairy Sci. 2000; 83: 1224-1227.
11. Gonlugur U, Akkurt I, Ozdemir L, Bakici M, Icagasioglu S. and Gultekin F; Antibiotic susceptibility patterns of respiratory isolates of *Staphylococcus aureus* in a Turkish university hospital. Zentralbl Veterinarmed, 2002; 49: 678-684.
12. Kiss G, Radvanyi S and Szigeti G; New combination for the therapy of canine otitis externa. I. Microbiology of otitis externa. J. Small Animal Practice, 1997; 38: 51-56.
13. Langer A and Nauriyal DS; Antibiogram pattern of bacterial isolates from subclinical mastitis in cows. Indian J. Field Vets., 2013; 9(1): 35-37.
14. Lee SH, Camargo CH, Gonçalves JL, Cruz AG, Sartori BT, Machado MB, Oliveira CA; Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. J Dairy Sci., 2012; 95: 7377-7383.
15. Lilenbaum W, Esteves AL and Souza GN; Prevalence and antimicrobial susceptibility of staphylococci isolated from saliva of clinically normal cats. Letters in Applied Microbiology, 1999; 28: 448-452.
16. Malik B; A Laboratory Manual of Veterinary Bacteriology, Mycology and Immnology, 1986; 2nd edn. CBS Publishers and Distributors, New Delhi, India, pp. 115-116.
17. Matos J, White D, Harmon R. and Langlois B; Isolation of *Staphylococcus aureus* from sites other than the lactating mammary gland. J. Dairy Sci., 1991; 74: 1544.
18. Medleau L, Brown RE and Miller WH; Frequency and antimicrobial susceptibility of Staphylococcus species isolated from canine pyodermas. Am. J. Vet. Res., 1986; 47: 229-231.
19. Misra P, Panda S and Misra S; Incidence and etiology of subclinical bovine mastitis in Orissa. Indian J. Anim. Hlth, 1973; 12: 175-180.
20. Mohan K; *Staphylococcus aureus* of human origin in udder infection of cow. Bulletin of Animal Health and Production in Africa, 1980; 28: 308-310.
21. Monsen T, Rönnmark M, Olofsson C et al.; An inexpensive and reliable method for routine identification of staphylococcal species. Eur. J. Clin. Microbiol. Infect. Dis., 1998; 17: 327-335.
22. Mundra V, Prasad A, Mahto D and Ahmad M; Isolation, identification and antibiogram of coagulase positive and negative staphylococci from bovine mastitis in Ranchi district. Indian J. Anim. Prod. Mgmt., 2009; 25(1-2): 72-75.
23. Mylotte J, Mcdermott C and Spooner J; Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteremia. Review Infect. Dis., 1987; 9: 891-907.
24. Pal M; Zoonoses,1997; 1st edn. R.M. Publishers, Delhi, India.
25. Pal M; Etiology and diagnosis of fungal mastitis in dairy animals. In Proc. symposium on "Mastitis and Milk Quality", 3rd IDFT Expo, 5th Sept. 2002, Pragati Maidan, New Delhi, India, p.217.
26. Pal M and Lee CW; Association of Prototheca with bovine mastitis. Korean J. Vet. Clin. Med., 1997; 14: 349-351.
27. Peles F, Wagner M, Varga L, Hein I, Rieck P, Gutser K, Keresztúri P, Kardos G, Turcsányi I, Béri B, Szabó A; Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. Int. J. Food Microbiol., 2007; 118: 186–193.
28. Pereira U, Oliveira D, Mesquita L, Costa G and Pereira L; Efficacy of *Staphylococcus aureus*

vaccines for bovine mastitis: a systematic review. Vet. Microbiol., 2011; 148: 117-124.
29. Roberson J, Fox L, Hancock D, Gay J. and Besser T; Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. J. Dairy Sci., 1994; 77: 3354-3364.
30. Roberson J, Fox L, Hancock D, Gay J and Besser T; Sources of intramammary infections from *Staphylococcus aureus* in dairy heifers at firs t parturition. J. Dairy Sci., 1998; 81: 687-693.
31. Roland P and Stroman D; Microbiology of acute otitis externa. Laryngoscope, 2002; 112: 1166-1177.
32. Ruzickova V; Characteristics of strains of *Staphylococcus aureus* isolated at dairy farms. Vet. Med. (Praha), 1994; 39: 37-44.
33. Sarma K; A Text book of Veterinary Bacteriology and Bacterial Diseases, 2002; 1st Edn., Kalyani Publishers, New Delhi, India, pp. 69-74.
34. Schultze W and Mercer H; Non-lactating cow therapy with a formulation of penicillin and novobiocin, therapeutic and prophylactic effects. Am. J. Vet. Res., 1976; 37: 1275.
35. Silva N; Identification and antimicrobial susceptibility patterns of Staphylococcus spp. isolated from canine chronic otitis externa. Arq. Bras. Med. Vet. Zootec., 2001; 53 (2): 138-143.
36. Suzuki K, Nishimura T, Baba S, Yanagita N and Ishigami H; Chronic suppurative otitis media. Clin. Infect. Dis., 2003; 24: 1066-1080.