

Comparative Herbal and Conventional Antimicrobial Susceptibility of Mammaliicoccus sciuri and M. lentus, Emerging Cause of Lethal Infections in Animals and Birds

Singh BR¹*, Chandra M², Agri H¹, Karthikeyan R¹, Yadav A¹, Jayakumar V¹

¹Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar-243 122, India. ²Department of Veterinary Microbiology, College of Veterinary Sciences, GADVASU, Ludhiana, Punjab, India.

Citation: Singh BR, Chandra M, Agri H, Karthikeyan R, Yadav A, Jayakumar V (2024). Comparative Herbal and Conventional Antimicrobial Susceptibility of *Mammaliicoccus sciuri and M. lentus*, Emerging Cause of Lethal Infections in Animals and Birds. *Acta Botanica Plantae.* **12 to 18. https://doi.org/10.51470/ABP.2024.03.03.12**

Corresponding Author: Singh BR | E-Mail: (brs1762@gmail.com)

Received 5 September 2024 | Revised 8 September 2024 | Accepted 4 October 2024 | Available Online 3 November 2024

Copyright: This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Strains of Mammalicoccus species are often considered as contaminants or at the most opportunistic pathogens but rarely as cause of infections. This retrospective data analytical study was undertaken to understand the role of Mammalicoccus species strains in the causation disease in humans, animals and birds. The data for the 12 years retrieved from Clinical Epidemiology laboratory of the Institute was analyzed statistically for determining the association of mammaliicocci and their antimicrobial resistance. The study revealed that M. lentus and M. sciuri species strains may cause lethal septicemia (14), abscesses and wounds (14), eye and ear infections (4), mastitis (7), metritis (5), prostatitis (1), upper respiratory tract (4) and urinary tract infections (2). Mammaliicoccus species strains caused septicemia in buffaloes, cattle, dog, elephant, spotted deer, tigers, poultry, and hawk besides causing other ailments in buffaloes, cattle, dogs, goat, horses, and humans. Most of the Mammaliicoccus strains were susceptible to thyme oil (94.03%), carvacrol (88.06%), ajowan oil (85.07%), and common antimicrobials used therapeutically including linezolid (100%), imipenem (94.03%) and tigecycline (92.54). Many of the Mammalicoccus strains were resistant to methicillin (37.31%) and vancomycin (35.82%), and 59.70% of the strains had multiple drug resistance (MDR). Strains isolated from heart blood of septicemic cases were significantly (p, <0.05) more resistant to ajowan oil, holy basil oil, cinnamaldehyde, carvacrol, carbapenems, macrolides, cefalosporins, tetracycline, ciprofloxacin and cefotaxime and were more often resistant to multiple antibiotics (p, < 0.01) than those associated with other infections. The Mammalicoccus strains of cattle origin were more often resistant (p, <0.05) to cinnamaldehyde, carvacrol, cinnamon oil, and macrolides (erythromycin/ azithromycin) and had MDR than those isolated from buffaloes, humans, and dogs. However, ESBL production was more common (p, <0.02) in Mammalicoccus isolates infecting buffaloes than those infecting horses. The study concluded that Mammalicoccus strains may cause a variety of infections in humans, animals and birds and possess resistance to methicillin, vancomycin along many high-end antibiotics.

Keywords: Methicillin-resistant Mammaliicoccus (MRM); Vancomycin-resistant Mammaliicoccus (VRM); ESBL; MDR; Carbapenem resistance; Tigecycline resistance; Septicemia; Mastitis

Introduction

There are five species in *Mammaliicoccus* genus including *M*. fleurettii, M. lentus, M. sciuri, M. stepanovicii, and M. vitulinus [1]. Mammaliicocci are frequently isolated as predominant Coagulase-Negatice Staphylococcus (CoNS) from animal sources (milk and meat) and are often considered harmless [2, 3] and rarely reported to be associated with mastitis in cows, illness in goats and piglets and from cases of canine dermatitis [4-8]. Strains of *M. sciuri* are also known to cause acne in humans [9]. Even some of *M. sciuri* isolates from goat milk are shown to be of probiotic value [10]. Mammaliicoccus strains are known to possess resistance genes to several antibiotics [4, 11-13]. In recent years, M. fleurettii, M. lentusand M. sciuri have been detected in cow milk in many countries [3, 7, 8]. Besides methicillin resistance in *M. sciri* isolates from dairy milk, on 17 dairy farms in Germany [8] among 64 isolates resistance phenotype to penicillin (58), cefoxitin (25), chloramphenicol (26), ciprofloxacin (25), clindamycin (49), erythromycin (17),

fusidic acid (61), gentamicin (8), kanamycin (9), linezolid (1), mupirocin (4), rifampicin (1), sulfamethoxazol (1), streptomycin (20), quinupristin/dalfopristin (26), tetracycline (37), tiamulin (59), and trimethoprim (30) were reported. Apart from milk, ground meats (beef, chicken, pork) has also been reported as the common source of drug-resistant mammaliicocci [2], and beta-lactam antibiotic-resistant M. sciuri and M. lentuswere detected from nasal swabs of cattle calves [7]. Methicillin resistance (mecA) gene reservoir M. sciuri was also reported from seabirds [14] but none of the two isolates of *M. sciuri* from the skin of the 41 healthy pigeons was resistant to ciprofloxacin, clindamycin, chloramphenicol, erythromycin, fosfomycin, gentamicin, levofloxacin, norfloxacin, rifampicin, tobramycin, trimethoprim/ sulfamethoxazole, vancomycin and not had multidrugresistance [15]. Methicillin-resistant *M. lentus* and *M. sciuri* are reported as the common nasal microflora in camels [16-18] and dromedary camels are suspected to be reservoirs of methicillinresistant Mammaliicoccus strains [18].

In a study on ruminants and camelids of New World [19], from 723 nasal swabs, M. sciuri, M. lentus and M. fleurettii strains were detected in 130, 43 and 11 swabs, respectively and many of the isolates had a multi-resistance phenotype. Mammaliicoccus sciuri strain causing acne [9] showed resistance penicillin but was susceptible to tetracycline, chloramphenicol, ciprofloxacin, levofloxacin, co-Trimoxazole, gentamicin, ofloxacin and aqueous extracts of herbs including Citruslimon, Psidiumguajava, Menthasachalinensis and Punicagranatum. Though mammaliicocci are frequently isolated from apparently healthy animals and their products [2, 3], very few reports are there of their isolation from clinically sick animals and humans except a few cases of mastitis, dermatitis, a few cases of illness in goats and pigs [4-8] and acne in humans [9], no precise information is available on their association with infections in humans, animals and birds in India. This study on comparative herbal and conventional antimicrobials susceptibility of Mammaliicoccus isolates from referred clinical samples of animals, humans, and birds, and also from samples of environmental, and apparently healthy humans and animals.

Materials and Methods

Samples and bacteriological analysis: Over the last 12 years samples from a total of 2555 referred clinical cases were received for bacteriological analysis in the Clinical Epidemiology laboratory of the department from veterinary poly clinic and Centre for Animal Disease Research and Diagnosis of the Institute. All the samples were processed following standard bacteria isolation and identification methods [20, 231] and isolates of *Mammaliicoccus* were confirmed based on their biochemical characteristics [22]. For further identification of *Mammaliicoccus* species isolates were confirmed matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) (Broker Daltonics Germany) profile. All the identified strains of *Mammaliicoccus* species were stored in glycerol medium at -80°C till tested and active cultures were stored on Nutrient agar slants at 4-8°C.

Antibiotic susceptibility assays: All the confirmed Mammaliicoccus species isolates were tested for their antimicrobial susceptibility using disk diffusion assay and results were interpreted in accordance with CLSI guidelines for Staphylococcus aureus [23]. All the antimicrobial disks (amoxicillin 50 μ g + clavulanic acid 10 μ g, ampicillin 10 μ g, azithromycin 15 µg, cefepime 10 µg, cefotaxime 10 µg, cefoxitin 30 µg, chloramphenicol 25 µg, ciprofloxacin 10 µg, cotrimoxazole 25 µg, erythromycin 15 µg, gentamicin 30 µg, imipenem 10 µg, linezolid 10 µg, meropenem 10 µg, nitrofurantoin 300 µg, tetracycline 30 µg, tigecycline 15 µg, and vancomycin 30 µg) were procured from BBL-Difco (USA). All tests were performed in triplicate for conformity on Mueller Hinton agar (BBL-Difco) plates. A methicillin-resistant and vancomycin-resistant (MRSA) strain (S. aureus ATCC 43300), a methicillin-susceptible vancomycin-resistant strain (S. aureus ATCC 29312) and a methicillin-susceptible vancomycinsusceptible strain (S. aureus MTCC 449) were used as controls in the study. The methicillin resistance was determined using cefoxitin-disk-diffusion assay (CDD) method [24] as per CLSI [23]. Vancomycin, imipenem, meropenem susceptibility and extended-spectrum β -lactamase production was confirmed and determined using respective E-tests (Biomerieux, India), and interpreted as per CLSI [23].

Herbal antimicrobial susceptibility assay: Herbal antimicrobial susceptibility was determined through disk diffusion assay [25] using disks (loaded with 2 mg of active ingredient) ajowan oil, guggul oil, holy basil oil (99% pure received from Subh Fragrance Ltd (Delhi), carvacrol, cinnamaldehyde, cinnamon oil, citral, lemongrass oil, thyme oil (95-99% pure from Sigma-Aldrich, USA) and sandalwood oil (100% pure from Mysore Sandal works (India). A measurable zone of bacterial growth inhibition around the disk was recorded as susceptible else resistant to the respective herbal antimicrobials.

Statistical analysis: All antimicrobial susceptibility data was entered in a Microsoft-Excel Sheet and analyzed for Odds ratio, and Chi-square analysis to find out any difference in susceptibility of Mammaliicoccus strains of different species and of different origins.

Results

Strains of Mammalicoccus species (M. lentus 43 and M. sciuri 24) were isolated from healthy humans carrying *M. sciuri* on finger nails (2), excreting bacteria in urine (M. sciuri 2, M. lentus 1), from buffen (M. lentus 2), in goat milk (M. lentus 1) and environmental sources (M. lentus 3, M. sciuri 3), and 53 isolates were from clinical samples of humans (from three of 625 samples) and animals (from 50 of 1930). Mammaliicoccus species strains were isolated from pus swabs of abscesses and wounds (14), swabs samples of eye and ear infections (4), milk of animals suffering from mastitis (2 buffaloes, 5 cows), uterine aspirates from cases of metritis (5), penile excretion of prostatitis cases (1), deep nasal swabs from cases of upper respiratory tract (4) infections and urine of patients with urinary tract infections (2). Mammaliicoccus species strains were also detected in pure culture from heart blood of 14 animals (2 buffaloes, 6 cattle, 1 dog, 1 elephant, 1 spotted deer and three tigers) and two birds (poultry, hawk) died due to septicemic infections (Tab. 1). Mammaliicoccus strains of two species (M. lentus, M. sciuri) were identified causing infections in many different species of animals and birds including buffalo (5 M. lentus, 2 M. sciuri), cattle (10 M. lentus, 1 M. sciuri), dogs (11 M. lentus, 4 M. sciuri 4), and elephant (1 M. lentus), goat (1 M. lentus), hawk (1 M. lentus), horses (4 M. lentus, 8 M. sciuri), humans (4 M. lentus, 4 M. sciuri), poultry bird (1M. sciuri), spotted deer (1 *M. sciuri*), and tigers (3 *M. lentus*).

Among the herbal antimicrobials thyme oil was the most effective, inhibiting 94.03% of the *Mammaliicoccus* strains in the study followed by carvacrol (88.06%), and ajowan oil (85.07%), and the least effective was guggul oil (28.36%), and lemongrass oil and sandalwood oil also inhibited less than 50% of the *Mammalicoccus* strains (Tab. 2). More than half (58.21%) of *Mammaliicoccus* strains were resistant to more than three herbal antimicrobials. Though there was no significant difference in herbal antimicrobial susceptibility of *M. lentus* and *M. sciuri* strains to most herbal antimicrobials, more of the *M. lentus* strains were resistant to cinnamaldehyde (OR 11.10; p, <0.01) than *M. sciuri* strains.

Among the antibiotics the most effective on *Mammaliicoccus* strains was linezolid inhibiting all the 67 strains followed by imipenem and tigecycline inhibiting 94.03% and 92.54% of the strains, respectively (Tab. 3). A total of 37.31% and 35.82% of *Mammalicoccus* species strains were methicillin resistant and vancomycin resistant, respectively but 59.70% of the strains were resistant to three or more classes of therapeutically used antibiotics (Tab. 4).

Though for most of the therapeutically used antibiotics no significant (p, >0.05) difference was evident among strains of M. *lentus* and *M. sciuri*, higher proportions of strains isolated from heart blood of animals died of septicemic infections were significantly (p, <0.05) more resistant to ajowan oil, holy basil oil, cinnamaldehyde, carvacrol, carbapenems, macrolides, cefalosporins, tetracycline, ciprofloxacin and cefotaxime and were more often resistant to multiple antibiotics (p, <0.01) than those identified as the cause of abscesses and wound infections. More of the Mammalicoccus isolates from cattle were resistant (p, <0.05) to cinnamaldehyde, carvacrol, cinnamon oil, and macrolides (erythromycin/ azithromycin) than from buffaloes, humans, and dogs. However, they were more often (p, <0.05)susceptible to vancomycin than Mammalicoccus strains causing infection in cattle and horses. Multiple herbal drug resistance was also more common (p, <0.05) in *Mammalicoccus* strains causing infection in cattle than those associated with infections in buffaloes, dogs, and horses. However, ESBL production was more common (p, <0.02) in Mammalicoccus isolates infecting buffaloes than those infecting horses. Multiple drug resistance was also more (p, <0.03) common in Mammalicoccus isolates from cattle than those from buffaloes, horses and humans. However, those Mammalicoccus causing infection in dogs had no significant difference in their MDR trait than those causing infections in other animals and humans but horses (p, <0.02).

Discussion

The present study revealed presence of *Mammalicoccus* species strains from healthy humans finger nails and urine of apparently healthy humans, from buffen (Cara-beef), goat milk, air, finger-print scanner surfaces, water, and plant leaves indicating environmental distribution and sustenance of Mammaliicoccus strains. Mammaliicoccus strains are reported earlier to thrive in commensalism in environment and on healthy animals and humans and also from milk and meats in different countries [2, 3] but rarely from India [26]. Isolation of *M. lentus* from goat milk seems to be an important finding as *M*. fleurettii, M. lentusand M. sciuri have been detected in cow milk in many countries [3, 7, 8, 27], and from fermented fish in India [26], but there is hardly any report of their isolation from goat milk. Though Mammalicoccus strains are known to occasionally cause mastitis in cows, illness in goats, piglets, canine dermatitis, and acne in humans [4-9] they are rarely reported to cause septicemia, abscesses and wounds, conjunctivitis, ear infections, metritis, prostatitis, upper respiratory tract and urinary tract infections earlier indicating the emerging importance of M. lentus and and M. sciuri in India as human and animal pathogens. Mammalicoccus lentus and M. sciuri were isolated as cause of mastitis from 4 (cows) and 3 cases (2 buffaloes and a cow), respectively. *Mammalicoccus lentus* and *M*. sciuri are also reported producing staphylococcal toxins responsible for toxic-shock syndrome too [27]. Though M. sciuri is known to cause mastitis in cows [5], M. lentus has rarely been reported to cause mastitis and mastitis in buffaloes by any Mammalicoccus strain is still rarer. Mammalicoccus species strains may probably be of zoonoticand nosocomial infection importance [28], and needs further well planned studies to understand their true potential as pathogens.

Of the 17 antibiotics tested, linezolid was the most effective antibiotic inhibiting all the isolates and four (chloramphenicol, imipenem, meropenem and tigecycline) more could inhibit more than 80% of the isolates. Observations are in concurrence to earlier observations on rarity of linezolid resistance among Gram-positive bacteria including staphylococci and mammalicocci [27, 29]. In rare cases, coaggulase-egative staphylococci are repoted resistant to linezolid due to mutation in 23sRNA genes [29]. Similarly, tigecycline resistance has also been been reported a rare phenomenon in staphylococci and mammalicocci occurring due to mutation in MepA efflux pump [30]. Among Gram-positive bacteria carbapenem resistance vary in differenerta genera but rare in *Micrococcaceae* members, some of the staphylococci may be intrinsically resistant and others may acquire resistance through mutations in penicillin-binding proteins (PBPs) are often considered responsible for carbapenem resistance [31]. Chloramphenicol is comparatively older broad-spectrum antibiotic and resistance in some of the M. sciuri strains has been reported to be plasmid mediated *cfr* gene [32].

In the present study multiple drug resistance detected in 59.7% of the strains is not surprising because *Mammaliicoccus* strains are reported to possess many resistance genes conferring resistance to several antibiotics [4, 11-13, 26, 27] including penicillin, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, rifampicin, sulfamethoxazol, streptomycin, quinupristin/dalfopristin, tetracycline, tiamulin, and trimethoprim in clinical strains and strains of food origin [2, 8].

Resistance to β -lactam antibiotics was detected in 22 strains (14 *M. lentus* and 8 *M. sciuri*) both from clinical (18) and non-clinical (4) samples and observations are in concurrence to earlier observations on mammaliicocci isolated from nasal swabs of cattle calves [7].

In the present study of the 67 Mammalicoccus strains 25 (16 M. lentus, 9 M. sciuri) strains were methicillin resistant (MR), in earlier studies birds are reported as reservoir of MR M. sciuri seabirds [14, 15] and MR strains of M. fleurettii, M. lentus and M sciuri are reported common in nasal microflora in camels [16-19]. The MR has also been seen in *M. sciuri* strains causing acne [9], however, in the present study none of the *M. sciuri* isolates from human samples was MR type but two strains of *M. lentus* showed resistance to methicillin. None of the 67 isolates of mammaliicocci had linezolid resistance but in an earlier study on cattle milk isolates showed that linezolid resistance may be there in mammaliicocci [8]. Reports of vancomycin resistance in mammaliicocci strains of clinical origin are rare but most of the strains of fish origin are reported vancomycin resistance [26]. In the present study, several *M. lentus* and *M. sciuri* strains isolated from clinical samples were resistant to vancomycin, and the finding is of public health concern.

The present study indicated that there was no significant difference in antimicrobial resistance mammaliicocci belonging to two different species but irrespective of the species strains isolated from cattle were more often resistant to multiple drugs and cattle may be considered as source of MDR strains of mammaliicocci as uspected in earlier studies [27]. Further the strains of *M. sciuri* and *M. lentus* associated septicemic infections were significantly (p, <0.05) more resistant many of the antimicrobials including ajowan oil, holy basil oil, cinnamaldehyde, carvacrol, carbapenems, macrolides, cefalosporins, tetracycline, ciprofloxacin, and cefotaxime than those detected in pus swabs of abscesses and wounds. The observations indicated that careful consideration is needed in the institution of antimicrobial therapy to cure different types of infections by *Mammalicoccus* strains.

The most potent herbal antimicrobial, thyme oil, inhibited

94.03% of the *Mammaliicoccus* followed by carvacrol (88.06%) and ajowan oil (85.07%). Rest of the herbal antimicrobial inhibited less than 80% of the *Mammaliicoccus* isolates, and the least effective was guggul oil which inhibited only 28.36% of the strains in the study indicating the presence of multiple herbal antimicrobial-resistant *M. sciuri* and *M. lentus* strains in India in animals, humans, birds and environment. Though in the last one decade emergence of herbal drug resistance has been reported in bacteria causing infections in animals and birds [33, 34], reports of herbal antimicrobial resistance in *Mammaliicoccus* strains are rare.

The study concluded that *Mammalicoccus* strains should not always be considered as contaminants as they can cause a variety in infections in humans and animals and even death due to lethal septicemia. Further, the common occurrence of methicillin and vancomycin resistance, MDR and resistance to cefalosporins and fourth generation β -lactam antibiotics in *M. lentus* and *M. sciuri* strains associated with clinical infections makes them the pathogen of concern. The study also indicated that herbal antimicrobials specially those having carvacrol as an active ingredients like thyme oil and ajaowan oil may be good alternatives to antibiotics against mammalicocci infections provided they can be administered safely.

Declaration of competing interest

None to declare.

Acknowledgments

The authors are thankful to the in-charge of the Clinical Epidemiology Laboratory, of the Division of Epidemiology, ICAR-ICAR-Indian Veterinary Research Institute, Izatnagar for providing access to data used in the study. The research work was supported by grants received from CAAST-ACLH (NAHEP/CAAST/2018-19) of ICAR-World Bank-funded National Agricultural Higher Education Project (NAHEP).

Tab. 1. Sources of isolation of Mar	nmaliicoccus species and assoc	ciation with different ailments
-------------------------------------	--------------------------------	---------------------------------

Common of commission	Sources of isolation of <i>Mammalicoccus</i> species isolates						
Source of samples	Mammaliicoccus sciuri (24)	Mammaliicoccus lentus (43)	cases				
Abscess and Wounds	Horses 8	Buffalo 1, dog 1, horses 3, human 1	14				
Eye infections	0	Dog 1	1				
Septicemic deaths	Dog 1, pig 1, spotted deer 1	Buffaloes 2, cattle, 6, elephant 1, hawk 1, tigers 3	16				
Ear infections	Dog 1	Dog 1, human 1	3				
Mastitis	Buffaloes 2, cattle 1	Cattle 4	7				
Metritis	0	Bitches 5	5				
Prostatitis	0	Dog 1	1				
Upper respiratory tract infection	Dogs 2	Dogs 2	4				
Urinary tract infections	0	Horse 1, human 1	2				
Air	Lab air 1	0	1				
Water	Pond water 1	Pond water 1	2				
Plants	Holy basil leaves 1	Holy basil leaves 1	2				
Meat	0	Buffalo meat 2	2				
Milk	0	Goat 1	1				
Finger print scanner 1	0	IVRI 1	1				
Carriers	On human nails 2	0	2				
Healthy excretors in urine	Humans 2	Human1	3				

 $Table.\,2.\,Herbal\,antimic robial\,drug\,susceptibility\,of\,Mammalic occus\,species\,is olates\,from\,different\,sources$

Types and sources of studies	Number of strains resistant to											
Types and sources of strains	AO	C	Cnh	CO	Ct	GO	HBO	LGO	SO	то		
Mammaliicoccus lentus (43)	9	8	14	12	17	29	23	23	24	3		
Mammaliicoccus sciuri (24)	1	0	1	2	11	19	8	11	12	1		
Abscess and wounds (14)	0	0	0	0	5	10	1	8	6	0		
Septicemic deaths (16)	4	5	8	7	9	13	11	7	8	0		
Buffalo (5 M. lentus, 2 M. sciuri)	0	0	0	0	2	5	2	2	2	0		
Cattle (10 M. lentus, 1 M. sciuri)	1	6	6	5	7	9	8	7	7	1		
Dogs (11 M. lentus, 4 M. sciuri)	1	0	2	3	6	9	7	8	9	0		
Elephant (1 M. lentus)	0	0	0	0	0	1	1	0	0	0		
Goat (1 M. lentus)	0	1	0	1	0	1	1	0	1	1		
Hawk (1 M. lentus)	1	0	1	0	1	1	1	1	1	0		
Horse (4 M. lentus, 8 M. sciuri)	1	0	1	1	5	9	1	7	6	0		
Humans (4 M. lentus, 4 M. sciuri)	2	0	0	0	3	5	4	4	4	1		
Poultry bird (1 M. sciuri)	0	0	0	0	1	0	1	1	0	0		
Spotted deer (1 M. sciuri)	0	0	0	0	0	1	1	0	0	0		
Tigers (3 M. lentus)	2	0	3	3	3	3	3	2	3	0		
Finger print scanners (1 M. lentus)	0	0	0	0	0	1	0	0	0	0		
Pond Water (1 M. lentus)	0	1	2	1	0	1	1	2	2	1		
Holy basil leaves (M. lentus1, M. sciuri 1)	2	0	0	0	0	2	0	0	1	0		
Laboratory air (1 M. sciuri)	0	0	0	0	0	0	0	0	0	0		
All (43 M. lentus, 24 M. sciuri)	10	8	15	14	28	48	31	34	36	4		
Percent resistant isolates	14.93	11.94	22.39	20.90	41.79	71.64	46.27	50.75	53.73	5.97		

AO, Ajowan oil; C, Carvacrol; Cnh, Cinnamaldehyde; CO, Cinnamon oil; Ct, Citral; GO, Guggul oil; HBO, Holy basil oil; LGO, Lemongrass oil; SO, Sandalwood oil; TO, Thyme oil

Table 3. Antimicrobial drug resistance in different Mammaliicoccus species strains of various origins

Tymos and		1				1	1						1	1			
sources of strains	AMC	Α	Az	Cfm	Ctx	C	Cf	Со	Е	G	Ι	L	м	Nft	Т	Tg	v
Mammaliicoccus lentus (43)	9	13	11	6	16	7	17	15	13	13	3	0	5	8	15	4	15
Mammaliicoccus sciuri (24)	6	5	10	8	7	1	5	6	10	10	1	0	2	9	8	1	9
Abscess and wounds (14)	3	3	2	2	2	0	2	3	3	3	0	0	0	2	3	0	5
Septicemic deaths (16)	6	4	9	6	10	3	8	5	8	6	2	0	4	6	9	3	4
Buffalo (5 M. lentus, 2 M. sciuri)	2	1	1	2	1	1	1	2	2	3	1	0	1	3	1	0	4
Cattle (10 M. lentus, 1 M. sciuri)	2	2	6	4	6	5	6	6	7	4	0	0	2	3	6	3	1
Dogs (<i>M. lentus</i> 11, <i>M. sciuri</i> 4)	4	6	3	4	5	2	7	5	4	6	1	0	2	2	2	0	6
Elephant (1, <i>M.</i> <i>lentus</i> 1)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Goat (<i>M. lentus</i> 1)	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1
Hawk (1, lentus 1)	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
Horse (<i>M. lentus</i> 4, <i>M. sciuri</i> 8)	2	2	2	2	2	0	1	4	3	3	0	0	0	2	4	0	6
Humans (<i>M. lentus</i> 4, <i>M. sciuri</i> 4)	0	2	2	0	0	0	1	2	2	2	0	0	0	0	2	1	1
Poultry bird (<i>M. sciuri</i> 1)	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	1	1
Spotted deer (<i>M. sciuri</i> 1)	1	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0
Tigers (<i>M. lentus</i> 3)	3	2	3	1	3	0	3	0	2	1	1	0	1	2	2	0	0
Finger print scanners (<i>M.</i> <i>lentus</i> 1)	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Pond Water (1, lentus)	1	2	1	1	2	0	2	2	1	1	1	0	1	1	2	0	2
Holy basil leaves (<i>M. lentus</i> 1, <i>M.</i> <i>sciuri</i> 1)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0
Laboratory air (<i>M. sciuri</i> 1)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
All resistant isolates	15	18	21	14	23	8	22	21	23	23	4	0	7	17	23	5	24
Percent resistant isolates	22.39	26.87	31.34	20.90	34.33	11.94	32.84	31.34	34.33	34.33	5.97	0.00	10.45	25.37	34.33	7.46	35.82

AMC, Amoxicillin + clavulanic acid; A, Ampicillin; Az, Azithromycin; Cfm, Cefepime; Ctx, Cefotaxime; C, Chloramphenicol; Cf, Ciprofloxacin; Co, Cotrimoxazole; E, Erythromycin; G, Gentamicin; I, Imipenem; L, Linezolid; M, Meropenem; Nft, Nitrofurantoin; T, Tetracycline; Tg, Tigecycline; V, Vancomycin

Table. 4. Different types of resistance in Mammaliicoccus species strains of various origins

Types and sources of strains	Number of strains having										
Types and sources of strains	MHDR	ESBL	CR	MaR	CFR	MR	MDR				
Mammaliicoccus lentus (43)	26	14	6	15	20	16	29				
Mammaliicoccus sciuri (24)	13	8	2	12	10	9	11				
Abscess and wounds (14)	5	3	0	3	4	3	3				
Septicemic deaths (16)	10	5	5	10	11	6	11				
Buffalo (5 <i>M. lentus,</i> 2 <i>M. sciuri</i>)	1	5	1	2	2	2	3				
Cattle (10 M. lentus, 1 M. sciuri)	10	3	2	9	7	4	10				
Dogs (M. lentus 11, M. sciuri 4)	8	5	2	4	8	6	11				
Elephant (1, <i>M. lentus</i> 1)	0	1	0	0	0	0	0				
Goat (M. lentus 1)	1	0	0	0	1	0	1				
Hawk (1, lentus 1)	1	0	0	0	1	0	1				
Horse (M. lentus 4, M. sciuri 8)	6	2	0	3	3	2	3				
Humans (<i>M. lentus</i> 4, <i>M. sciuri</i> 4)	5	2	0	2	1	4	3				
Poultry bird (<i>M. sciuri</i> 1)	1	1	0	1	1	0	1				
Spotted deer (M. sciuri 1)	0	0	0	1	0	1	1				
Tigers (M. lentus 3)	3	1	2	3	3	3	3				
Finger print scanners (<i>M. lentus</i> 1)	0	1	0	0	1	1	1				
Pond Water (1, lentus)	2	1	1	1	2	2	2				
Holy basil leaves (<i>M. lentus</i> 1, <i>M. sciuri</i> 1)	1	0	0	0	0	0	0				
Laboratory air (<i>M. sciuri</i> 1)	0	0	0	1	0	0	0				
All	39	22	8	27	30	25	40				

MHDR, Multiple herbal drug-resistant; ESBL, Extended spectrum β -lactamase production; CR, Carbapenem-resistant; MaR, Macrolide-resistant; CFR, Cefalosporin-resistant; MR, Methicillin-resistant by cefoxitin (30 μ g) disk diffusion assay method; MDR, Multiple antimicrobial drug resistant

References

- 1. Madhaiyan M, Wirth JS, Saravanan VS. Phylogenomic analyses of the Staphylococcaceae family suggest the reclassification of five species within the genus *Staphylococcus* as heterotypic synonyms, the promotion of five subspecies to novel species, the taxonomic reassignment of five *Staphylococcus* species to *Mammaliicoccus* gen. nov., and the formal assignment of *Nosocomiicoccus* to the family Staphylococcaceae. Int J Syst Evol Microbiol. 2020; 70: 5926–5936.
- 2. Osada M, Aung MS, Urushibara N, Kawaguchiya M, Ohashi N, Hirose M, et al. Prevalence and antimicrobial resistance of *Staphylococcus aureus* and coagulase-negative *Staphylococcus/Mammaliicoccus* from retail ground meat: Identification of broad genetic diversity in fosfomycin resistance gene *fos*B. Pathogens 2022; 11:469.
- Goncalves JL, Mani R, Sreevatsan S, Ruegg PL. Apparent prevalence and selected risk factors of methicillin-resistant *Staphylococcus aureus* and non-aureus staphylococci and mammaliicocci in bulk tank milk of dairy herds in Indiana, Ohio, and Michigan. JDS Communications. 2023; 4:489–495.
- 4. Nemeghaire S, Argudín MA, Feßler AT, Hauschild T, Schwarz S, Butaye P. The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. Vet Microbiol. 2014; 171: 342–356.
- 5. Khazandi M, Al-Farha AA, Coombs GW, O'Dea M, Pang S, Trott DJ, et al. Genomic characterization of coagulasenegative staphylococci including methicillin-resistant *Staphylococcus sciuri* causing bovine mastitis. Vet Microbiol. 2018; 219: 17–22.
- Loncaric I, Lepuschitz S, Ruppitsch W, Trstan A, Andreadis T, Bouchlis N, et al. Increased genetic diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from companion animals. Vet Microbiol. 2019; 235: 118-126.
- 7. Schnitt A, Lienen T,Wichmann-Schauer H, Tenhagen BA. The occurrence of methicillin-resistant non-aureus staphylococci in samples from cows, young stock, and the environment on German dairy farms. J Dairy Sci. 2021; 104: 4604–4614.
- 8. Lienen T, Schnitt A, Hammerl JA, Maurischat S, Tenhagen BA. *Mammaliicoccus* spp. from German dairy farms exhibit a wide range of antimicrobial resistance genes and non-wildtype phenotypes to several antibiotic classes. Biology (Basel).2022; 11(2):152.
- 9. Goswami M. Antibacterial activity of some medicinal plants and antibiotics against *Staphylococcus aureus* and *Mammaliicoccus sciuri* isolated from acne. Biomed Biotechnol Res J (BBRJ). 2022; 6(3): 372-38.
- 10. Naqqash T, Wazir N, Aslam K, Shabir G, Tahir M, ShaikhRS. First report on the probiotic potential of *Mammaliicoccus sciuri* isolated from raw goat milk. Biosci Microbiota,Food Hlth. 2022; 41(4): 149–159.

- 11. Harrison EM, Paterson GK, Holden MTG, Ba X, Rolo J, Morgan FJE, et al. A novel hybrid SCC*mec-mec*C region in *Staphylococcus sciuri*. J Antimicrob Chemother. 2013; 69: 911–918.
- 12. Paterson GK. Genomic epidemiology of methicillinresistant *Staphylococcus sciuri* carrying a *SCCmec-mec*C hybrid element. Infect Genet Evol. 2020; 79: 104148.
- Schwendener S, Perreten V. The *bla* and *mec* families of βlactam resistance genes in the genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*: An in-depth analysis with emphasis on *Macrococcus*. J Antimicrobial Chemother. 2022; 77 (7):1796–1827.
- 14. Saraiva MMS, de Leon C, Silva N, Raso TF, Serafini PP, Givisiez PEN, et al. *Staphylococcus sciuri* as a reservoir of *mecA* to *Staphylococcus aureus* in non-migratory seabirds from a Remote Oceanic Island. Microb Drug Resist. 2021; 27:553–561.
- Szczuka E, Wesołowska M, Krawiec A, Kosicki JZ. Staphylococcal species composition in the skin microbiota of domestic pigeons (*Columba livia domestica*). PLoS ONE. 2023; 18(7):e0287261.
- 16. Akarsu H, Liljander A, Younan M, Brodard I, Overesch G, Glücks I, et al. Genomic characterization and antimicrobial susceptibility of Dromedary-associated Staphylococcaceae from the Horn of Africa. Appl Environ Microbiol. 2022; 88: e01146-22.
- 17. Silva V, Caniça M, Manageiro V, Verbisck N, Tejedor-Junco MT, González-Martin M et al. *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci in nostrils and buccal mucosa of healthy camels used for recreational purposes. Animals. 2022; 12: 10.
- 18. Belhout C, Boyen F, Vereecke N, Theuns S, Taibi N, Stegger M, de la Fé-Rodríguez PY, Bouayad L, Elgroud R, Butaye P. Prevalence and Molecular Characterization of Methicillin-Resistant Staphylococci (MRS) and Mammaliicocci (MRM) in Dromedary Camels from Algeria: First Detection of SCCmec-mecC Hybrid in Methicillin-Resistant Mammaliicoccus lentus. Antibiotics. 2023; 12(4):674. https://doi.org/10.3390/antibiotics12040674
- 19. Schauer B, Szostak MP, Ehricht R, Monecke S, Feßler AT, Schwarz S, et al. Diversity of methicillin-resistant coagulase-negative *Staphylococcus* spp. and methicillinresistant *Mammaliicoccus* spp. isolated from ruminants and New World camelids. Vet Microbiol. 2021; 254:109005.
- Carter GR. Diagnostic Procedures in Veterinary Microbiology. 2nd edn. Springfield: Charles C Thomas Publishers, 1975.
 Singh BR. Labtop for Microbiology Laboratory. Berlin: Lambert Academic Publishing, AG & Co. 2009.
- 21. Singh BR. Labtop for Microbiology Laboratory. Berlin: Lambert Academic Publishing, AG & Co. 2009.

- 22. Brenner DJ, Krieg NR, Staley JT, Garrity GM. Bergey's Manual of Systematic Bacteriology, Vol 2: The Proteobacteria, Part B: The Gammaproteobacteria. New York: Bergey's Manual Trust, Springer. 2005.
- 23. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th edn. CLSI supplement M100. Wayne: Clinical and Laboratory Standards Institute. 2017.
- 24. Alipour F, Ahmadi M, Javadi S. Evaluation of different methods to detect methicillin resistance in *Staphylococcus aureus* (MRSA). J Infect Pbl Hlth. 2014; 7(3): 186-191.
- 25. Singh BR, Singh V, Singh RK, Ebibeni N. Antimicrobial activity of lemongrass (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin. Intl Res J Pharmacy Pharmacol. 2011; 1: 228-236.
- Majumdar RK, Gupta S. Isolation, identification and characterization of *Staphylococcus* sp. from Indian ethnic fermented fish product. Lett Appl Microbiol. 2020; 71(4):359-368. doi: 10.1111/lam.13362.
- Nemeghaire S, Argudí MA, Feßler AT, Hauschild T, Schwarz S, Butaye P. The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. Vet Microbiol. (2014). 171(3-4), 342-356. <u>https://doi.org/10.1016/j.vetmic.</u> 2014.02.005
- 28. Shaker MN, Hmdan TA, Issa AH. Isolation and diagnosis of *Staphylococcus lentus* from different operation theatre hospitals. Sci J Med Res. 2018; 2 (8): 177-181.

- 29. Stefani S, Bongiorno D, Mongelli G, Campanile F. Linezolid resistance in staphylococci. *Pharmaceuticals*, 2010; *3*(7): 1988.
- 30. Huang H,Wan P, Luo X, Lu Y, Li X, Xiong W, Zeng Z. Tigecycline resistance-associated mutations in the MepA efflux pump in *Staphylococcus aureus*. Microbiol Spectr. 2023; 11:e00634-23.
- 31. Aurilio C, Sanson P, Barbarisi M, Pota V, Giaccari LG, Coppolino F, Barbarisi A, Passavanti MB, Pace MC. Mechanisms of action of carbapenem resistance. A n t i b i o t i c s . 2 0 2 2 ; 1 1 (3) : 4 2 1 . https://doi.org/10.3390/antibiotics11030421.
- 32. Schwarz S, Werckenthin C, Kehrenberg C. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. Antimicrob Agents Chemother. 2000; 44(9): 2530-2533.
- 33. Vadhana P, Singh BR, Bhardwaj M, Singh SV. Emergence of herbal antimicrobial drug resistance in clinical bacterial isolates. Pharm Anal Acta. 2015; 6: 434.
- Bhardwaj M, Singh BR, Sinha DK, Vadhana P, Vinodhkumar OR, Singh SV, et al. Potential of herbal drug and antibiotic combination therapy: A new approach to treat multidrugresistant bacteria. Pharmaceutica Analytica Acta. 2016; 7(11):1-4.