

Research Article

# Carriage Rate of Coagulase Negative Staphylococci in a Rural Human Population with or without Companion Livestock

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DOI: <https://doi.org/10.24321/0019.5138.201930>

## I N F O

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### How to cite this article:

Jangra S, Sharma M, Chakravarti A, Chattopadhyaya D. Carriage Rate of Coagulase Negative Staphylococci in a Rural Human Population with or without Companion Livestock. *J Commun Dis* 2019; 51(4): 1-9.

Date of Submission: 2019-10-04

Date of Acceptance: 2020-01-28

## A B S T R A C T

**Background:** The emergence of antibiotic resistance in Coagulase Negative Staphylococci (CoNS) in both humans and companion livestock has been recognized to be an issue of public health concern. There are limited studies reported to evaluate the risk of transmission of antibiotic resistant CoNS from companion livestock to their human owners.

**Material & Methods:** A random of 200 households, 100 each with or without companion livestock were included in the study (sub-grouped as Sgr Ia and Sgr Ib resp.). All the selected subjects were sampled from different anatomical sites, CoNS were identified by standard procedures and subjected to AST.

**Results:** Out of a total 400 and 440 samples from Sgr Ia and Sgr Ib, 232 (58%) and 162 (37%) resp. showed positive isolation for CoNS with *S.epidermidis* as the most common isolated species. Two species of CoNS viz. *S.scuiri* and *S.warneri* were also isolated from Sgr Ia subjects alone. Methicillin resistance was found to be high among all the CoNS isolates. Resistance rates towards non- $\beta$  lactam antibiotics were found to be significantly higher among Sgr Ia compared to Sgr Ib.

**Conclusion:** The present study suggests that transmission of various species as well as resistance genes can be possible from companion livestock to their owners. Hence human population in rural community with companion livestock should be routinely monitored for acquisition of antimicrobial resistance so as to prevent the further spread to human community.

**Keywords:** CoNS, Antimicrobial Resistance, Carriage Rate, Companion Livestock, Rural Population

## Introduction

Coagulase-Negative Staphylococci (CoNS), considered as contaminants earlier, are now being recognized to be

associated with many serious infections in human e.g. native valve endocarditis, peritonitis, surgical sites infections, prosthetic devices and shunt infections.<sup>1,2</sup> CoNS consist of

a variety of staphylococcus species some of which such as *S. epidermidis* and *S. haemolyticus* colonize permanently or transiently at the anterior nares, skin and mucous membranes and act as source bacteremia and other infections.<sup>3</sup> The situation is further complicated by emergence of resistance to common antimicrobial agents among them.<sup>4</sup> While most of the reports on CoNS as human pathogens are hospital based, those on the prevalence of CoNS in a community are limited.<sup>5,6</sup> Determining the carriage rate of CoNS in a community, specially the multi-drug resistant strains, could reflect existence of the reservoir pool of such organisms in the community.<sup>7</sup>

In the rural community in North India majority of the population human beings are engaged in farming as profession. There are several risk factors for them that could be correlated for acquisition of drug resistant CoNS such as contact with animals carrying them due to indiscriminate use of antibiotics in animals.<sup>8</sup> There are few studies on prevalence of CoNS in healthy companion animals from developed countries like the United States, where dogs and cats have been considered as companion animals.<sup>9,10</sup> However in a developing country like India, the livestock e.g. buffaloes and cattle also represent companion animals in rural population due to sharing of common residential premises with humans. Some species of CoNS are known to be exclusively associated with animals.<sup>11</sup> Therefore, search for such strains in human population with companion livestock could be indicative of their transmission probability from livestock to human population. The present study was undertaken to find out the carriage rate of CoNS, identification of species, their antibiogram pattern and possible risk factors for acquisition in a rural population with livestock as companion animal.

## Materials and Methods

### Study Area

The study was carried out in two villages in the district Gurugram, Haryana state, India with the population in the villages being predominantly farmers having livestock as companions sharing the same residential premises with that of the owner. Ethical clearance was obtained from independent institutional ethical committee for this study.

### Selection of Study Population

- Selection of human subjects (grouped as Group I or Gr I): A random of 200 households, comprising of 100 each with or without companion livestock (cattle and buffalo) were selected for the survey. All the human subjects (ranging 2-6 members/household), regardless of age and sex, residing in two categories of households i.e. with or without companion livestock, grouped as subgroup Ia (Sgr Ia) and subgroup Ib (Sgr Ib) subjects respectively were sampled.

- Selection of companion livestock (grouped as Group II or Gr II): All the livestock sharing residential premises with the Sgr Ia subjects (ranging 3-6 livestock/household) were selected for sampling.
- The selected human subjects, were provided patient information sheet and consent was obtained from them for the study and only those members consenting to participate in the study and agreed for sampling from themselves as well as from companion livestock were included.

### Exclusion Criteria

The following categories of human subjects and companion animals were excluded from the study.

- History of antibiotics therapy, ongoing or within past 4 weeks.
- Any wound infection.
- Any major surgery in recent past (within 1 month).
- Close contacts with hospital environment.

### Collection of Demographic and Epidemiological Information

Information regarding age, sex, level of education, number of companion livestock and duration of their association were collected from the human subjects employing a predesigned proforma.

### Selection of Sites for Sampling

Samples were taken from 5 anatomical sites viz. skin on hands, skin on feet, anterior nares, axilla and head in case of human subjects while in case of companion livestock samples were taken from 4 anatomical sites viz. muzzle, udder, skin, and inner nares.<sup>12</sup>

### Sample Collection and Transport

#### Sample Collection

##### 1. Human Subjects

- **Skin of hands and feet:** Cotton swabs, moistened with sterile physiological saline, were used, one for each hand. The swab on each hand was swiped on the dorsum of hand to cover all the fingers, including finger-rings (if worn by the participants), tip of nails and inter-digital spaces. The swabs from each hand were then pooled into one tube containing transport medium. Similar procedure was followed for swabbing dorsum of feet covering areas on toes, toe nails and inter-digital spaces.<sup>13</sup>
- **Anterior nares:** The swab, premoistened with sterile normal saline was inserted approximately 2 cm into both the nares, turn by turn and rotated against the anterior nasal mucosa for 3 seconds.
- **Axilla:** Skin on the axillary area on both sides were

rubbed in a rotating manner to cover up to a 5cm × 5cm area approximately using separate swab for each side.

## 2. Companion Livestock

Swab samples from companion livestock were collected by the same procedure as for human individuals with the help of their handlers from the following anatomical sites.

- **Muzzle:** Inside both the nostrils (5 to 10 cm deep).
- **Skin:** Area on the skin between the thigh on both sides and udder.
- **Perineum:** Area on the skin in the groin area.
- **Udder:** Surface of udder in an area of 5 cm × 5 cm approx.

### Sample Transport

The collected swab samples were transported to the Microbiology laboratory in Phosphate Buffer Saline (pH 7.2-7.4) as transport medium with icepack within 1-2 hours.

### Processing of Samples and Identification of CoNS Isolates

Each swab sample was streaked on 5% sheep blood agar and inoculated plates were incubated aerobically at 37°C overnight (18-24 hrs). Presumptive staphylococcal colonies were stained with gram stain and were subjected to catalase test and further evaluated for confirmation of coagulase production by tube coagulase test using human plasma.<sup>14</sup> All resulting coagulase negative staphylococcal colonies were further identified to species level using the Vitek 2c gram positive identification cards according to manufacturer's

directions (Biomerieux India Pvt. Ltd.).

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of CoNS isolates were determined by Kirby Bauer agar disc diffusion method recommended by Clinical and Laboratory Standard Institutes (CLSI, 2018). Antimicrobial agents tested and their respective strengths were as follows:

Penicillin (10 U), Amikacin (30 µg), Erythromycin (15 µg), Clindamycin (2 µg), Tetracycline (30 µg), cefoxitin (30 µg), cefuroxime (30 µg), Vancomycin (30 µg), Ampicillin (10 µg), Ciprofloxacin (5 µg). Methicillin resistance was detected by using Cefoxitin (30 µg) disc as a surrogate marker for methicillin resistance.<sup>15</sup>

### Statistical Analysis

All the data collected from selected population were analyzed by using Chi-square test which is an accepted tool for categorical variables. Association of positivity rate among sub parameters of Sgr Ia subjects were determined by using Chi square for trend.<sup>16</sup>

### Result

Out of 100 households each in Sgr Ia and Sgr Ib, total of 400 and 440 human subjects respectively were sampled while all the companion livestock (sharing same residential premises with Sgr Ia human subjects), totaling to 320 animals were sampled.

**Table I. Carriage rate of CoNS in human subjects with or without companion livestock in relation to various parameters**

Demographic and epidemiological parameter		Positivity rate for CoNS				Statistical analysis
		Sgr Ia		Sgr Ib		
		No of subjects (n=400)	No (%)	No of subjects (n=440)	No (%)	
Age group (years)	0-15	76	19 (25)	110	49 (11.1)	$\chi^2=7.40, p=0.007^*$
	16-45	223	179 (80)	209	64 (30.6)	$\chi^2=108, p=0.000^*$
	>45	101	34 (33.6)	121	49 (30)	$\chi^2=1.09, p=NS(0.12)^*$
Sex	Male	208	108 (51.9)	194	87 (45.9)	$\chi^2=2.01, p=NS(0.15)^*$
	Female	192	92 (47.9)	246	130 (53)	$\chi^2=1.04, p=NS(0.36)^*$
Companion livestock size	1	46	10 (21.7)	NA		$\chi^2=63.4, p<0.01^{**}$
	2-5	54	15 (27.7)			
	>5	300	207 (69)			
Duration of association with companion livestock (years)	0-5	90	35 (38.8)	NA		$\chi^2=23.4, p<0.001^{**}$
	6-10	110	58 (52.7)			
	>10	200	139 (69.5)			

NA-Not Applicable.

\* Statistical comparison between Sgr Ia and Sgr Ib for the same parameter.

\*\*Chi square trend herd size and duration of association with increase in positivity rate.

**Table 2. Carriage rate of CoNS according to anatomical sites among human subjects with or without companion livestock and companion livestock**

Sites (Human subjects)	Sites (companion livestock)	Group I Human subjects				Group II Companion Livestock		Statistical Analysis*
		Sgr Ia		Sgr Ib		No of samples	Carriage rate No (%)	
		No of samples	Carriage rate No (%)	No of samples	Carriage rate No (%)			
Axilla		400	240 (60)	440	160 (36.3)	NA		$\chi^2=46$ ; $p=0.00$
Skin (foot)		400	208 (52)	440	148 (33.5)	NA		$\chi^2=28$ ; $p=0.00$
Anterior nares		400	160 (40)	440	132 (30)	NA		$\chi^2=9.23$ ; $p=NS(0.12)$
Skin (hands)	Skin	400	320 (80)	440	220 (50)	320	80 (25)	$\chi^2= 82$ ; $p=0.00$
	Muzzle	NA		NA		320	250 (78)	NA
	Perineum	NA		NA		320	112 (35)	NA
	Udder	NA		NA		320	211 (66.5)	NA
Total		1600	928 (58)	1760	660 (37.5)	1280	653 (51.1)	$\chi^2=141$ ; $p<0.001$

NA=Not Applicable

\*Statistical comparison between human subjects belonging to the groups Sgr Ia and Sgr Ib.

A total of 232 subjects out of 400 subjects in Sgr Ia and 162 out of 440 subjects in Sgr Ib yielded positive isolation for CoNS with the prevalence rate of 58% and 37 % respectively. Subjects in age group of 16-45 years showed higher CoNS positivity rate compared to other age groups in Sgr Ia subjects whereas no statistically difference was observed in the carriage rate among subjects of Sgr Ib belonging to various age groups.

No statistically significant difference was observed in positivity rate between males and females belonging to Sgr Ia and Sgr Ib subjects. Positivity rate was found to be higher in the families who had a greater number of companion livestock (>5) with them. CoNS positivity was found to be higher in the families who reported living with companion animals for more than 10 years compared to those with lesser duration of association (Table 1). A total of 163 out of 320 companion livestock sampled in Gr II yielded positive isolation for CoNS (data not shown in table).

Isolation rate of CoNS among humans from various anatomical sites were found to be significantly higher in Sgr Ia compared to the corresponding anatomical sites in Sgr Ib except from anterior nares. Difference in isolation rate between Sgr Ia and Sgr Ib subjects was more marked for samples collected from the skin on hands. Companion livestock showed a higher isolation rate of CoNS from muzzle area compared to other anatomical sites (Table 2).

*S.epidermidis* was found to be the most prevalent species among CoNS isolates from humans regardless of association with companion livestock in this study. Relative carriage rate of various species of CoNS among companion livestock was found to be similar as that of the human subjects associated with them However two species viz, *S.scuiri* and *S.warneri* which are predominantly associated with animals were also isolated from Sgr Ia individuals which were associated with handling of companions livestock (Table 3).

**Table 3. Relative distribution of various Species of CoNS among human subjects (Sgr Ia and Sgr Ib) and companion livestock (Gr II)**

Species	Gr I (Human)		Gr II (Companion livestock)
	Sgr Ia (n=928)	Sgr Ib (n=660)	(n=410)
<i>S.epidermidis</i>	250 (26.9%)*	230 (34.8%)*	136 (32.9%)
<i>S.hemolyticus</i>	111 (11.9%)	94 (14.2%)	74 (18%)
<i>S.hominis</i>	222 (23.9%)	143 (21.6%)	61 (15%)
<i>S.capitis</i>	150 (16.2%)	193 (29.2%)	-----
<i>S.scuiri</i>	111 (11.9%)	----	78 (19%)
<i>S.warneri</i>	84 (9%)	----	61 (15.1%)

\*Significantly higher rate ( $p<0.05$ ) compared to other species in the same group (Sgr Ia and Sgr Ib).

Table 4. Antibiotic resistance profile of the CoNS isolates

Group		Source of CoNS isolate	No (%) Resistant strains									
			P	E	CD	TC	CX	CXM	Cip	Amc	Amp	Ak
Gr I (human)	Sgr Ia	928	538 (58)	139 (15)	167 (18)	232 (25)	372 (40)	334 (36)	186 (20)	47 (5)	61 (6.5)	19 (2.5)
	Sgr Ib	660	240 (40)	30 (5)	30 (5)	42 (7)	60 (10)	120 (20)	24 (4)	0	0	0
Statistical analysis*		$\chi^2$	72.00	44	64	93	187,	59	90,	14,	19,	10,
		p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gr II (Animal)		410	287 (70)	103 (25)	115 (28)	164 (40)	174 (42.5)	82 (20)	125 (30.5)	22.5 (5.5)	47 (11.5)	20 (5)
Statistical analysis **		$\chi^2$	17.4	19	17,	40	0.6	70	70	0.05	0.9	8.1
		p	0.00	0.00	0.00	0.00	NS (0.4)	0.00	0.00	NS (0.8)	NS (0.7)	NS (0.05)

P-Penicillin, E-Erythromycin, CD-Clindamycin, TC-Tetracycline, CX-Cefoxitine, CXM-Cefuroxime, Cip-Ciprofloxacin, Amc-Amoxyclave, Amp-Ampicillin, Ak-Amikacin.

\*Statistical comparison between human subjects with (Sgr Ia) and without companion livestock (Sgr Ib).

\*\*Statistical analysis between Sgr Ia (human subjects) and Gr II.

Methicillin Resistant CoNS (MRCoNS) rate among isolates from Sgr Ia were found to be higher compared to Sgr Ib (Table 4). while among companion livestock's (Gr. II) prevalence of MRCoNS was also found to be high. Sgr Ia humans showed statistically significant higher rate of resistance towards non  $\beta$  lactam antibiotics including erythromycin, clindamycin, ciprofloxacin as compared to Sgr Ib humans. Companion livestock group II carried a higher resistance to penicillin along with an overall resistance to the non  $\beta$  lactam antimicrobials whereas the resistance pattern was same for human subjects of Sgr Ia. Regarding the non  $\beta$  lactam antibiotics the resistance rates were found to be higher among companion livestock similar to the human subjects belonging to Sgr Ia compared to Sgr Ib human subjects (Table 4).

## Discussion

Rural human community associated with animals are at increased risk of exposure to many zoonotic pathogens e.g., Staphylococcus, Streptococcus, *Escherichia coli*, *Shigella spp.* following which they could also become carriers and can spread infections to the community.<sup>17</sup> In the rural community in India companion livestock are considered as family members and close proximity or direct animal contact can be seen in many households (companion livestock). This leads to the potential risk of transmission of a multitude of pathogenic microorganisms, including multidrug-resistant bacteria, between companion livestock and their human owners.<sup>18,19</sup> Higher prevalence of CoNS among human subjects in the present study associated with companion livestock compared to those without

such association suggest their possible acquisition from companion livestock sharing the same premises.

High carriage rate of CoNS in the age group between 15-45 years could be related to greater involvement of this particular age group in day to day care of the companion livestock e.g. feeding, milking bathing etc. This was further evident from the lack of association of CoNS positivity with any particular age in sub group of human subjects without companion livestock. Shinde et al in their study on adoption of improved dairy practices by dairy farmers also found that majority (65%) of the respondents were in middle age group (31-45 years), followed by young age group up to 30 years (20%) and remaining and remaining 15% in old age group (46 years and above).<sup>20</sup> There are numerous studies in India that showed that both male and females are equally engaged in routine activities involving care of livestock with woman spending about two hours a day on animal rearing activities associated with the care of companion livestock supporting our observation of equal distribution of CoNS positivity in both sexes.<sup>21,22</sup> Based on a data from labour inputs, from rural Haryana and West Bengal it was shown that in each rural households, in India it is reported that predominantly women perform all the day to day activities related to caring, feeding, cleaning, health and production of livestock.<sup>23</sup> while some activities such as vaccinations, deworming, grazing, purchase of fodder and medicines, and taking animals to the dispensary are performed predominantly by male members, providing equal chances for acquisition of pathogenic organisms among both male and females from animals.<sup>24</sup> According to a study by Fang et al the carriage rate of staphylococcus in

large-scale farmers (those who were in animal farming for a long time) was found to be significantly higher than that in small-scale farmers suggesting an association between herd size and duration with companion livestock with CoNS as observed in the present study.<sup>25</sup>

There are several studies reported on various species of CoNS associated with various anatomical sites. Vanessa M et al found *S.epidermidis* to be the most common species isolated from the nares, perineum, inguinal skin, axillae and interdigital skin of human beings in her study.<sup>26</sup> Another study by Rogers et al on human subjects with dogs as companion animal showed *S.epidermidis* to be the most common (52%) species of CoNS isolated predominantly from the nasal cavity of dogs with the similar species of CoNS species isolated from similar sites in humans.<sup>27</sup> Animals are found to be common reservoirs of CoNS and carry CoNS on their skin, noses, udders, upper alimentary and urogenital tract and intestinal tract and thus may be transmitted to human due to contact with those animal by hands during routine activities like milking, bathing etc.<sup>7</sup> High isolation rate of staphylococci from hands in our study is an expected observation as hands are reported to be principle vector for transmitting microorganisms between pets and human subjects.

*S.epidermidis* was found to be the most prevalent species among all the CoNS isolates that can cause significant disease in this study. A study by Ibrahim et al also showed the predominant species of CoNS isolated and identified from various body sites were *S.epidermidis* (54.7%) while other species of CoNS were isolated less frequently i.e., *S.haemolyticus* 23.4%, *S.hominis* 5.8% followed by *S.lugdunensis* in 4%, *S.capitis* in 3.6% and *S.saprophyticus* in 3.1%.<sup>28</sup> The other salient observations in the present study was that some species of CoNS e.g., *S.scuri* and *S.warneri* that are reported to be predominantly associated with animals were also found in human subjects associated with them (Sgr Ia) in the present study strengthening further the possibility of transmission of these CoNS species from their companion livestock.<sup>29</sup>

Transmission of resistance from animals to humans can take place through a variety of routes while the food-borne route probably is the most important route for enteric bacterial pathogens, such as *Salmonella enterica*, *Campylobacter coli/jejuni* and *Yersinia enterocolitica*, for other resistant pathogens, e.g., MRSA direct contact between animal and humans may be the major route of transmission (e.g. MRSA CC398). Researchers have shown that transfer of multiple resistant *Staphylococcus intermedius* and quinolone resistant *Campylobacter jejuni* can occur between humans and dogs living in the same household).<sup>30</sup> Growth promoters are used in livestock as they are used as food animals although use of antimicrobials for growth promotions is not

a prevalent practice in India where livestock are not used as food animals in the country. However, in India there is little regulation on the use of drugs in veterinary sector for various ailments in animals resulting in the indiscriminate use of antibiotics in companion livestock that might lead to development of high antimicrobial resistance in them.<sup>31</sup> Bovine mastitis is an expensive disease affecting lactating cattle's in India. Some studies from India reported that the incidence of sub clinical mastitis ranged from 19.2-83% in cows. About 70-80% economic loss has been attributed due to sub clinical mastitis.<sup>32</sup> In addition nearly all dairy cows receive intramammary infusions of prophylactic doses of antibiotics following each lactation to prevent and control future mastitis-primarily with penicillin's, cephalosporins, or other beta-lactam drugs.<sup>33</sup> Antimicrobial resistance of mastitis pathogens has received a lot of interest in the past few years causing heavy economic burden in dairy sector attributable to bacteria resistant to routinely used antibiotics.<sup>34</sup> There is very little data available on resistance among CoNS in healthy human population although there are numerous reports on antimicrobial resistance among various species of CoNS as carrier associated with various human infections. An epidemiological study of staphylococci among healthy humans (without companion livestock) and its molecular characterization showed MR-CoNS rate 54.2% along with *S.epidermidis* as their most common species among all CoNS isolates.<sup>35</sup> Whereas the prevalence of MR-CoNS carriage among adult Americans were found to be 51% in 2006 and 47% in 2008 with most prevalent species being *S.epidermidis*, which contrasts with previous community-based surveys, where the reported rates ranged from 11 to 30% for methicillin resistance.<sup>36</sup> Adabi et al. showed 71.7% of school students in India harbored CoNS in their nasal cavity with 16.7% among the isolates being MRCoNS.<sup>37</sup> Jonathan H et al. from Leeds, UK in their study showed most of the healthy individuals carrying CoNS, the organisms were resistant to penicillin, tetracycline and erythromycin and the most commonly species encountered was *S.epidermidis*.<sup>38</sup> The antibiotics that were found to be most commonly co-resistant with CoNS in our study was tetracycline. This is in contrast to the study by Elena G et al. who reported a high rate of methicillin resistance, 81.3% with all MRCoNS isolates that were methicillin resistant also resistant to tetracycline. However in the present study we found lower degree of association between MRCoNS and tetracycline with 25% of CoNS were co-resistant to tetracycline, showed that 74% of clinically healthy dogs harbored CoNS in their nares, from which 23.7%, 16.9%, 5.1% were resistant to penicillin, erythromycin and tetracycline respectively.<sup>39</sup> Huber et al. from Switzerland showed *S.scuri* as most prevalent species among all the CoNS isolates from which nasal MRCoNS rate was observed as 67% and 60.2% among pig farmers and

veterinarians respectively that was comparatively higher than the present study.<sup>40</sup>

Admittedly however we could not perform molecular analysis of the CoNS isolated from human and companion animals to provide more convincing evidence of transmission animal - human Nevertheless along with an higher isolation rate among human subjects with companion livestock compared to those without companion animals, isolation of two species among the farmer population i.e., *S. scuiri* and *S. warneri* that is identical to human subjects associated with them provide evidence that human population in association with companion animals are at increased risk for acquisition of multidrug resistant CoNS that may further spread horizontally in community. To the best of our knowledge there is not much literature on carriage of CoNS in healthy population in association with livestock in India.

### Conclusion

The present study suggests that transmission of various species as well as resistance genes can be possible from companion livestock to their owners associated with activities related to care of them. Hence human population in rural community with companion livestock should be routinely monitored for acquisition of antimicrobial resistance so as to prevent the further spread to human community.

**Conflict of Interest:** None

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