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Prevalence and colonization of *S. aureus* in nasal cavity of domestic animals and of those who are taking care of them

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Abstract

Staphylococci can infect both mammals and humans; they are one of the most common bacterial pathogens and one of the most important factors for nosocomial infections. Therefore, the purpose of this study was to compare human and animal samples in relation to *Staphylococci* and to examine their resistance to antibiotics in Thessaly, Greece.

Method: A total of 344 samples were collected; 74 from humans and 270 from domestic animals (cats, dogs, goats, horses, sheep). Sampling was performed using cotton swabs. All *Staphylococci* were isolated, identified and tested for their susceptibility to various antimicrobial agents using an automated system.

Result: The majority of the samples collected were positive to a bacterium. The bacterium, which was isolated was mainly *Staphylococcus sciuri*. The isolates of *S. aureus* were detected in the human samples, as well as in the samples from dogs, and horses. Regarding the resistance to antibiotics, the highest rates were recorded against penicillin. In conclusion, humans, domestic animals and people who are taking care of them carried *S. aureus*.

Novelty of the work

The bacterium, which was isolated to a greater extent was *Staphylococcus sciuri* (12,2%). A really high percentage of Staphylococcus spp was found in human and in dog. Thus, we can conclude that there is a close contact between human and dog which allows the transmission of Staphylococcus.spp between them. The isolates of *S. aureus* were detected only in the samples of the human, dog, horse. Regarding the resistance to antibiotics, the highest rates were recorded against penicillin. The resistant rate of cat and dog to penicillin was the highest recorded (100%). The resistance rate of goat was also remarkable (20%). Moreover, pig, was resistant to all antibiotics apart from kanamycin, in which it was susceptible. Most of these animals' owners were asked and told that the animals had been given antibiotics at the moment in which the samples were taken or even earlier. These high rates of resistance, therefore, may occur due to the excessive use of penicillin that had been given by vets as a therapy to these animals. *S. aureus* isolates exhibited resistance to oxacillin, cefoxitin and penicillin.

Introduction

Until now, 47 species of bacteria belonging to the genus Staphylococcus have been identified, which can affect both humans and other kinds of mammals. The bacteria isolated in most cases from humans are *S.aureus* and *Staphylococcus epidermidis* [1-3]. *S.aureus* is the most pathogenic of the Staphylococcus genus [4,5]. It is characterized by its high resistance to antibiotics [6,7].

Bacteria of the Staphylococcus genus can also cause food poisoning, and *S. aureus* is the major factor. *S. aureus* food poisoning incidents are the most common food-borne diseases worldwide [8,9]. The aim of this research is to examine the nasal carriage of *S. aureus* in domestic animals and the people involved in their care in Thessaly, Greece

Material and method

Sample collection and S. aureus identification

The survey was conducted in 2016 on a total of 344 samples, 74 of which were from humans and the remaining 270 from domestic animals such as cats, dogs, goats, horses and sheep. All samples were collected from houses that bred and kept domesticated animals and from the people who were taking care of them in Thessaly, Greece.

Sampling was performed using cotton swabs, while samples were collected from the participants' nasal cavity. The laboratory experiment

was performed in the Microbiology Department of the University Hospital of Thessaly, Larissa.

The swab of each coating was initially inoculated into fresh nutrient broth (Tryptic Soy Broth) at 37°C for 24 hours in order to enrich the sample. A new cultivation of the broth in blood agar containing 5% sheep blood followed. From each culture, one or more colonies with different morphology (size, colour, haemolysis etc) were sub-cultured and were collected for further analysis.

Gram staining and catalase production was performed in all bacteria isolates. Gram-positive cocci, catalase-positive were selected. Identification to species level and antibiotic susceptibility testing was performed using an automated system.

Result

A total of 196 out of 344 samples (57.0%) were positive for Staphylococcus spp. 94.6% of horse samples studied were positive to

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one or more different staphylococcal species, as well as 60.8% of human samples (Table 1). The most common isolate which was identified was *Staphylococcus sciuri* (12.2%), followed by *S. aureus* and *Staphylococcus lentus* (5.6% for each other) (Table 2). *S. intermedius* was found in a higher percentage in cats, *S. aureus* in dogs, humans and horses, *S. lentus* in goats and *S. sciuri* in sheep (Table 3).

A major difference was demonstrated in the rates of susceptibility to clindamycin, fusidic acid, penicillin, cefoxitin and tetracycline. In particular, in sheep and pigs the rates of sensitivity to clindamycin were the lowest ones, while the highest ones were demonstrated to cats, dogs and horses (100%). Similarly, in sheep and pigs the rates of susceptibility to fusidic acid, were the lowest ones, whereas the highest ones were demonstrated to cats, dogs and horses (100%). Moreover, the goats and the horses were more susceptible to penicillin, the pigs where less susceptible to cefoxitin and to tetracycline (Table 4).

S. sciuri: All the samples of *S. sciuri*, that were examined, were susceptible to cefoxitin and tetracycline. Also, 91.3% of the samples were susceptible to erythromycin, 9.1% to clindamycin, none of them to fusidic acid, 8.7% to penicillin and 87.0% to oxacillin.

S. aureus: All *S. aureus* samples were susceptible to erythromycin, clindamycin, fusidic acid, tetracycline and kanamycin. Half of the samples were resistant to penicillin and 75% of the samples were susceptible to oxacillin and cefoxitin.

Table 1. Number of samples studied and positive samples to Staphylococcus spp

	Number of samples	Number of positive samples	Rate of positive samples
Human	74	45	60,8
Sheep	117	40	34,2
Goat	49	42	85,7
Dog	33	10	30,3
Cat	22	14	63,6
Horse	37	35	94,6
Pig	37	10	27,0
Total	344	196	57,0

Table 2. Data regarding the positive samples to Staphylococcus spp

		n	%
	Cat	14	7,1
Origin	Dog	10	5,1
	Goat	42	21,4
Origin	Horse	35	17,9
ong	Human	45	23,0
	Pig	10	5,1
	Sheep	40	20,4
Catalase	No	None	None
Catalase	Yes	195	100,0
<i>C</i> 1	No	100	52,9
Coagulase	Yes	89	47,1
	Not found	129	65,8
	Aerococcus viridans	1	0,5
	CoNS	6	3,1
	Kocuria kristinae	1	0,5
	S. aureus	11	5,6
I.I	S. epidermidis	1	0,5
Identification	S. gallinarum	3	1,5
	S. lentus	11	5,6
	S. sciuri	24	12,2
	S. simulans	1	0,5
	S. xylosus	5	2,6
	S.intermedius	3	1,5

S.epidermidis: The *S. epidermidis* sample was susceptible to clindamycin, penicillin, oxacillin, cefoxitin and tetracycline.

S.lentus: All *S. lentus* samples were susceptible to oxacillin and cefoxitin. Moreover, 60.0% of the samples were susceptible to erythromycin, 66.7% to clindamycin, 90.0% to fusidic acid, 80.0% to penicillin and tetracycline.

S. intermedius: All S. intermedius samples tested were susceptible to erythromycin, clindamycin, fusidic acid, oxacillin, cefoxitin and kanamycin. Finally, 33.3% of the samples were susceptible to tetracycline and all samples were resistant to penicillin.

S.xylosus:All samples of Staphylococcus xylosus tested were susceptible to clindamycin, penicillin, oxacillin and cefoxitin.

S.simulans: The *Staphylococcus simulans* sample was susceptible to all antibiotics apart from kanamycin in which it was not tested (Table 4-12).

Discussion

57% of the samples were positive to Staphylococcus.spp, while 8 of the 11 identified bacteria belonged to the Staphylococcus genus. The most common strain identified was *S.sciuri* (12.2%), followed by *S.aureus* and *S.lentus* (5.6% for each other) (Tables 1 and 2). Although most of the samples were taken from sheep, these animals showed less (34.2%) infected samples than horses (94.6%), humans (60.8%) and goats (85.7%) (Table 1). The above results are consistent with the results of the research by Loeffler et al [10] where the most isolated bacteria were *S. aureus* and *S. intemedius*.

The rate of samples found infected with *S.aureus* (5.6%) is close to the respective percentage found in a survey conducted in Tunisia, where 6.5% of the samples carried the particular bacterium [11].Higher rates of positive samples have been recorded in the literature, such as in the research by Gömez-Sanz et al [12], where *S.aureus* strains were recorded in 12% of the samples. In addition, strains of *S.aureus* were found in 20% of dog samples, 17,8 of human samples and only 2.9% of horse samples (Table 3). The results are quite similar to other surveys, that were carried out in different countries, as shown in Table 5.

As shown in the literature, various percentages of positive samples have been recorded. Differences from survey to survey are expected and may be due to both the different number of samples and the different region or time period (Figure 1). Experiments performed to determine the positivity of the samples to catalase and to coagulase, showed that all samples were positive to catalase (100%), while 47,1% of samples were positive to both catalase and coagulase. 65,8% of samples was not identified (Table 2). The lowest rates of coagulation positive samples were found in horses (22.9%) and the highest ones in cats (70%) and sheep (67.5%). 42% (human), 44,4% (dog) and 59.5% (goat) of the positive samples were positive to coagulase (Table 3 and Figure 2). Similar results were also shown by two more studies, where the percentage of samples from human was approximately 40%, while those from animals were 33-41%. A small deviation was found in dog samples and more than 30% deviation was found in cat samples [10-12]. Regarding the susceptibility of different samples to antibiotics, the rates recorded were quite high (47-100%). The highest rate was found against kanamycin, while 94,7% and 89,7% of the samples tested for their susceptibility to cefoxitin and oxacillin respectively were found susceptible to the corresponding antibiotic. 53,4% of the samples were resistant to penicillin. A significant difference was observed in the clindamycin, fusidic acid, penicillin and terracycline susceptibility

1 (2,2)

None

1 (2,2)

1 (2,2)

None

1 (10)

None

None

None

None

None

1 (2,5)

1 (2,5)

20 (50)

None

1 (2,5)

None

		Origin						
		Cat	Dog	Goat	Horse	Human	Pig	Sheep
		n (%)	n (%)	n (%)	n(%)	n (%)	n(%)	n(%)
Constant	No	3 (30,0)	5 (55,6)	17 (40,5)	27 (77,1)	25 (58,1)	10 (100)	13 (32,5)
Coagulase	Yes	7 (70,0)	4 (44,4)	25 (59,5)	8 (22,9)	18 (41,9)	None	27 (67,5)
	Not found	10 (71,4)	8 (80)	25 (59,5)	33 (94,3)	27 (60)	9 (90)	17 (42,5)
	Aerococcus viridans	None	None	None	None	1 (2,2)	None	None
	CoNS	None	None	None	None	6 (13,3)	None	None
	Kocuria kristinae	None	None	None	1 (2,9)	None	None	None
	S. aureus	None	2 (20)	None	1 (2,9)	8 (17,8)	None	None

None

1 (2,4)

8 (19)

3 (7,1)

1 (2,4)

4 (9,5)

None

None

None

None

None

None

None

None

Table 3. Percentages of positive coagulase samples and identification of samples depending on their origin

Table 4. Resistance of samples to different antibiotics, depending on their origin

None

1 (7,1)

None

None

None

None

3 (21,4)

S. epidermidis

S. gallinarum

S. lentus

S. sciuri

S. simulans

S. xylosus

S.intermedius

Identification

		Origin							
		Cat	Dog	Goat	Horse	Human	Pig	Sheep	P Fisher's exac test
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	lest
	Susceptibility	3 (100)	2 (100)	13 (65)	2 (100)	6 (75)	None	21 (95,5)	0,130
Erythromycin	Intermediate	None	None	1 (5)	None	1 (12,5)	None	None	
	Resistance	None	None	6 (30)	None	1 (12,5)	1 (100)	1 (4,5)	
	Susceptibility	3 (100)	2 (100)	13 (68,4)	2 (100)	7 (87,5)	None	4 (19)	0,001
Clindamycin	Intermediate	None	None	5 (26,3)	None	1 (12,5)	None	15 (71,4)	
	Resistance	None	None	1 (5,3)	None	None	1 (100)	2 (9,5)	
	Susceptibility	3 (100)	2 (100)	13 (65)	2 (100)	6 (75)	None	2 (9,1)	<0,001
Fusidic acid	Intermediate	None	None	6 (30)	None	2 (25)	1 (100)	18 (81,8)	
	Resistance	None	None	1 (5)	None	None	None	2 (9,1)	
	Susceptibility	None	None	16 (80)	2 (100)	5 (62,5)	None	4 (18,2)	<0,001
Penicillin	Intermediate	None	0 (0)	0 (0)	None	None	None	None	
	Resistance	3 (100)	2 (100)	4 (20)	None	3 (37,5)	1 (100)	18 (81,8)	
	Susceptibility	3 (100)	2 (100)	20 (100)	2 (100)	6 (75)	None	19 (86,4)	0,074
Oxacillin	Intermediate	None	None	None	None	None	None	None	
	Resistance	None	None	None	None	2 (25)	1 (100)	3 (13,6)	
	Susceptibility	3 (100)	2 (100)	20 (100)	2 (100)	6 (75)	none	21 (100)	0,009
Cefoxitin	Intermediate	None	None	None	None	None	None	None	
	Resistance	None	None	None	None	2 (25)	1 (100)	None	
	Susceptibility	1 (33,3)	2 (100)	13 (65)	2 (100)	7 (87,5)	None	21 (95,5)	0,015
Tetracycline	Intermediate	None	None	None	None	None	None	None	
	Resistance	2 (66,7)	None	7 (35)	None	1 (12,5)	1 (100)	1 (4,5)	
	Susceptibility	1 (100)	1 (100)	5 (100)	2 (100)	5 (100)	1 (100)	3 (100)	_*
Kanamycin	Intermediate	None	None	None	None	None	None	None	
	Resistance	None	None	None	None	None	None	None	

*not calculated

Table 5. Rates of contaminated samples per organism by S. aureus. Comparison with other surveys

Research	Dog	Human	Cat	Horse
Current Research/ Greece	20%	17,8%	0%	2,9%
Faires/Canada [13]	8,3%	18%	10%	-
Hanselman/ -Canada [14]	68%	27%	57%	-
Drougka/Greece [15]	37%	38,9%	30%	-
Weese/Canada [16]	-	13%	-	4,7%
Gómez-Sanzetal/Spain [12]	9,3%	41,8%	25%	-
El-Jakee/Egypt [17]	16%	20%	-	-
Vincze/ Germany [18]	5,8%	-	12,2%	22,2%
Abdel-moein al/Egypt [19]	4,5%	3,6%	0%	-

P Fisher's exact

test

<0,001

<0,001

	Susceptibility	Intermediate	Resistance
	n (%)	n (%)	n (%)
Erythromycin	6 (60,0)	None	4 (40,0)
Clindamycin	6 (66,7)	3 (33,3)	None
Fusidic acid	9 (90,0)	1 (10)	None
Penicillin	8 (80,0)	None	2 (20,0)
Oxacillin	10 (100,0)	None	None
Cefoxitin	10 (100,0)	None	None
Tetracycline	8 (80,0)	None	2 (20,0)
Kanamycin	None	None	None

Table 6. Resistance of *S.lentus* samples to different antibiotics (n=11)

 Table 7. Resistance of S. epidermidids samples to different antibiotics(n=1)

	Susceptibility	Intermediate	Resistance	
	n (%)	n (%)	n (%)	
Erythromycin	None	1 (100,0)	None	
Clindamycin	1 (100,0)	None	None	
Fusidic acid	None	1 (100,0)	None	
Penicillin	1 (100,0)	None	None	
Oxacillin	1 (100,0)	None	None	
Cefocitin	1 (100,0)	None	None	
Tetracycline	1 (100,0)	None	None	
Kanamycin	None	None	None	

Table 8. Resistance of S.aureus samples to different antibiotics(n=11)

	Susceptibility	Intermediate	Resistance	
	n (%)	n (%)	n (%)	
Erythromycin	8 (100,0)	None	None	
Clindamycin	8 (100,0)	None	None	
Fusidic acid	8 (100,0)	None	0 (0,0)	
Penicillin	4 (50,0)	None	4 (50,0)	
Oxacillin	6 (75,0)	None	2 (25,0)	
Cefoxitin	6 (75,0)	None	2 (25,0)	
Tetracycline	8 (100,0)	None	None	
Kanamycin	7 (100,0)	None	None	

Table 9. Resistance of *S.sciuri* samples to different antibiotics (n=24)

	Susceptibility	Intermediate	Resistance
	n (%)	n (%)	n (%)
Erythromycin	21 (91,3)	1 (4,3)	1 (4,3)
Clindamycin	2 (9,1)	18 (81,8)	2 (9,1)
Fusidic acid	None	21 (91,3)	2 (8,7)
Penicillin	2 (8,7)	None	21 (91,3)
Oxacillin	20 (87,0)	None	3 (13)
Cefoxitin	22 (100,0)	None	None
Tetracycline	23 (100,0)	None	None
Kanamycin	2 (100,0)	None	None

Table 10. Resistance of S.simulans samples to different antibiotics (n=1)

	Susceptibility	Intermediate	Resistance
	n (%)	n (%)	n (%)
Erythromycin	1 (100,0)	None	None
Clindamycin	1 (100,0)	None	None
Fusidic acid	1 (100,0)	None	None
Penicillin	1 (100,0)	None	None
Oxacillin	1 (100,0)	None	None
Cefoxitin	1 (100,0)	None	None
Tetracycline	1 (100,0)	None	None
Kanamycin	None	None	None

Table 11. Resistance of S.xylosus samples to different antibiotics(n=5)

	Susceptibility	Intermediate	Resistance
	n (%)	n (%)	n (%)
Erythromycin	4 (80,0)	None	1 (20,0)
Clindamycin	5 (100,0)	None	None
Fusidic acid	1 (20,0)	3 (60,0)	1 (20,0)
Penicillin	5 (100,0)	None	None
Oxacillin	5 (100,0)	None	None
Cefoxitin	5 (100,0)	None	None
Tetracycline	2 (40,0)	None	3 (60,0)
Kanamycin	1 (100,0)	None	None

Table 12. Resistance of S.intermedius samples to different antibiotics(n=3)

	Susceptibility	Intermediate	Resistance
	n (%)	n (%)	n (%)
Erythromycin	3 (100,0)	None	None
Clindamycin	3 (100,0)	None	None
Fusidic acid	3 (100,0)	None	None
Penicillin	None	None	3 (100,0)
Oxacillin	3 (100,0)	None	None
Cefoxitin	3 (100,0)	None	None
Tetracycline	1 (33,3)	None	2 (66,7)
Kanamycin	1 (100,0)	None	None

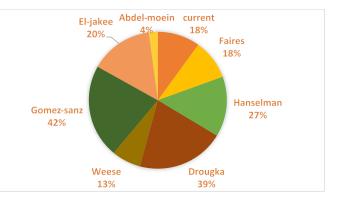


Figure 1. Percentages of contaminated human samples from S. aureus- Comparison between different surveys

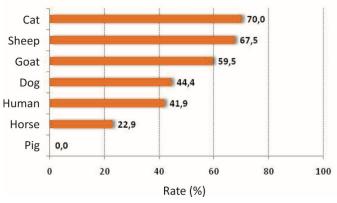


Figure 2. Rates of positive samples in coagulase

rates. In particular, the susceptibility rate of sheep to clindamycin was the lowest recorded, while the highest occurred in cats, dogs and horses. The sheep susceptibility rate to fusidic acid was lower than that of the cat, dog and horse. Goats and horses were more susceptible to penicillin comparing with other animals (Table 4 and Figure 3).

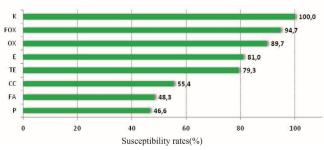


Figure 3. Susceptibility rates of samples to different antibiotics

Regarding the bacteria, S. epidermidis samples were sensitive against clindamycin, penicillin, oxacillin, cefoxitin and tetracycline antibiotics, while all S. lentus samples were susceptible to oxacillin and cefoxitin substances. 60% of the S.lentus samples showed sensitivity to erythromycin, 66.7% to clindamycin, 90% to fusidic acid, and 80% to penicillin and tetracycline. Staphylococcus aureus strains were sensitive to erythromycin, clindamycin, fusidic acid, tetracycline and kanamycin. Half of them were susceptible to penicillin and 75% to oxacillin and cefoxitin. S.sciuri strains were susceptible to cefoxitin and tetracycline. 91.3% of S.sciuri samples were sensitive to erythromycin, 87.0% of them, to oxacillin and only 9.1% and 8.7% to clindamycin and penicillin respectively. The sample that was containing S. simulans was susceptible to all antibiotics apart from kanamycin, in which it was not tested. Strains of S. xylosus were susceptible to penicillin, clindamycin, oxacillin and cefoxitin and kanamycin. 20.0% of the S.xylosus samples were susceptible to fusidic acid and 40.0% to tetracycline. Finally, strains of S.intermedius were found to be sensitive to erythromycin, clindamycin, fusidic acid, oxacillin and cefoxitin. In addition, 33.3% of the S.intermedius samples were sensitive to tetracycline and all samples were resistant to penicillin (Tables 6-12). Generally speaking, S.aureus demonstrated high rates of susceptibility and sensitivity to the most antibiotics that were used, a data which is similar with the results of other surveys. Resistance to other antibiotics such as oxytetracycline, enroflaxin, methicillin, erythromycin, amoxicillin and clindamycin was demonstrated in quite high rates. The antibiotics in which S.aureus was more susceptible were tetracycline, ciprofloxacin, cefoxerazone and cefoxitin.

Conclusion

S.aureus is one of the most commonly corresponding bacteria in humans and animals, while new research is being carried out for a better and more direct addressing to its health impacts on organisms. The results indicated that Staphylococcus strains were detected in the samples, with *S. sciuri* occurring as the most common strain, while *S.aureus* was identified only in dogs, horses and humans. The antibiotic against which the highest levels of resistance from both *S.aureus* and other *Staphylococci* were recorded was penicillin, however, *S.aureus* showed susceptibility to most antibiotics. Further studies should be conducted to provide additional information on the ecology of *S.aureus* in both humans and domestic animals.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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