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Co-Habitation of *Staphylococcus lugdunensis* with *Staphylococcus aureus* Resistant to Methicillin and Vancomycin in the Nasal Snares of Laboratory Rats

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Abstract

The public health problem created by multidrug resistant bacteria in the 21st century continues to receive attention by researchers all over the world. As the production of new antibiotics is not commeasurable with the rate of evolvement of MDR bacteria, the news of a proposed new antibiotic "Lugdunin" is much awaited and a welcomed development. Lugdunin is produced by *Staphylococcus lugdunensis* and has the ability to kill *S. aureus*. Both bacteria are nasal colonizers. The present investigation looks into the antibiotic susceptibility pattern of co-habitation of *S. lugdunensis* with methicillin and vancomycin resistant *Staphylococcus aureus* in laboratory bred Wister rats. Nasal swabs of anaesthetized rats were collected using a sterile cotton swab moistened in 0.9% saline solution. All swabs were inoculated into nutrient broth, cultured at 37˚C for 24 hrs. Overnight bacterial growth plated on blood agar and incubated at 37˚C for 24 hrs. Organism identification and antibiotic susceptibility test were by using BioMerieux VITEK 2 compact automated system (BioMerieux, Marcy I'Etoile France), according to the manufacturers guidelines. Results obtained showed co-habitation of *S. aureus* with co-agulase negative bacteria, inclusive of *S. lugdunensis*. All the isolates were resistant to methicillin with a 33.3% resistance to vancomycin. The difference between the number of antibiotic resistant or sensitive varied statistically among the Staphylococcal isolates. For *S. aureus* 1, the difference was significant with p-value 0.034 but not significant for isolates 2, 3 and 4 with p-values of 0.158, 0.477 and 0.158 respectively. A statistically significant difference was seen with *S. lugdunensis*. The result from the study therefore, showed that the colonization of the nasal snares of the laboratory bred rats with *S. aureus* and other co-agulase negative Staphylococci was not affected by the presence of *S. lugdunensis*.

Keywords

Nasal, *Staphylococcus lugdunensis*, *Staphylococcus aureus*, Methicillin,

Vancomycin Resistance, Rats

1. Introduction

The staphylococcus genus is made up of coagulase positive *Staphylococcus aureus* and a wide range of coagulase negative staphylococci. Both coagulase positive and coagulase negative Staphylococcus can be found inhabiting the human skin and mucosae [1]. The human nose is said to be home to more than 50 bacterial species, inclusive of *S. aureus* and coagulase negative Staphylococci [2]. Also, a notable feature of these staphylococci species is their ability to become resistant to antimicrobials such as methicillin and vancomycin [3]. Some staphylococci are opportunistic pathogens; while others such as the coagulase positive *S. aureus* which is of clinical importance have received much attention in recent years by researchers due to the emergence of strains resistant to methicillin [4] [5]. Nasal carriage of methicillin resistant to strains of *S. aureus* has also been reported with such carriages being said to vary among individuals [1] [2]. It is postulated that about 30% of human nasal passages are colonized with *S. aureus* while the remaining 70% of humans' show no signs of colonization [2]. Due to the burden caused by the resistance of *S. aureus* to methicillin, there has been worldwide attention given to the bacterium in recent years with the aim of searching for means by which the problem of multidrug resistant to bacteria (MDR), inclusive of MRSA can be curtailed. The search for a solution continues as new drugs are not being manufactured fast enough. Recently, however, attention is drawn to a proposed new antibiotic "Lugdunin" reported to be a product from *Staphylococcus lugdunensis*. This bacterium that can also be found colonising the nasal snares is said to have the capability to kill *S. aureus*, as has been reported by various researchers [6] [7] [8]. *S. lugdunensis* is said to produce lugdunin a novel thiazolidine-containing cycle peptide antibiotic that acts in inhibiting colonisation of *S. aureus* [6]. These researchers suggested that lugdunin producing *S. lugdunensis* could be valuable in preventing Staphylococcal infections as well as being effective against strains of MRSA. Zipper *et al.* [6], indicated that it was not yet clear how lugdunin worked, but postulated the bacterium to be a powerful enemy of *S. aureus*.

However, humans are not the only ones affected by this bacteria super bug; but the colonisation with MRSA in animals has also received a worldwide attention [9]. Since the first report of MRSA, researchers have isolated the bacterium from livestock intended for human consumption as well as from meat produce [10]. Also, the emergence of MRSA in other animals such as horses and pet animals has raised questions on the probability of a human origin as well as questions being asked on the host specificity of MRSA [11]. From the first cattle outbreak reported by De Vriese *et al.* [12], there have been sporadic reports of animal cases in different parts of the world, from North America [13] to Germany [14] as well as in other parts of the world. In most of the reported cases, however, MRSA infections were found to have been associated with a variety of infections ranging from bacteremia, septic arthritis, skin and soft tissue infections to implant infections [15]. Reports indicated that MRSA had also been isolated

from human companion animals. Rich *et al.* [16] first reported MRSA emergence in animals that were companions to humans, with the strains being linked to hospital acquired MRSA (haMRSA) in the UK, and thus suggested an origin from humans. However, there are reports that the human companion strains are different from those isolated from livestock and meat production animals [9]. The presence of MRSA has also been established in cats, horses and rabbits by researchers [17] [18]. The risk factors for MRSA acquisition by these animals are said to mirror those of humans [9] and this includes factors such as the use of antimicrobials, aminoglycosides amongst others [19].

With the advent of emerging and re-emerging infectious diseases worldwide, the role of zoonosis cannot be ignored. Studies have shown that apart from pets being reservoirs to MRSA and other staphylococci infections, there is the high risk carriage amongst visionary staff and owners of animal pets despite their not having direct hospital contacts [20]. As the world struggles to tackle the public health problem created by emerging MDR strains of bacteria, there will be the need to look into undetected colonised animals who serve as reservoirs for the continuous propagation of infections in humans [21] [22]. This might help out in the sustainability of new drugs when produced such as in the case of the proposed new antibiotic "lugdunin". Antibiotics are not being produced as fast as they are needed to tackle new emerging bacterial strains. As the world is running out of ideas on new antibiotics, all new findings that would lead to the production of new antibiotics would be useful. The present investigation looks into the antimicrobial susceptibility pattern exhibited by coagulase positive *S. aureus* and coagulase negative *Staphylococcus lugdunensis* as well as other coagulase negative Staphylococcus found co-habiting and isolated from nasal snares of laboratory bred rats. This is with a view of highlighting this co-habitation of methicillin resistant *S. aureus* and *S. lugdunensis*. The findings might help drug manufacturers in their quest for the search of new antibiotics to tackle the problem of MRSA and the likes.

2. Materials and Methods

2.1. Experimental Animals

Twelve weeks, old male white Albino Wistar rats weighing 100 - 120 g were obtained from Experimental Animal Care Center located in the College of Pharmacy, King Saud University Riyadh. The rats were housed under standard, suitable conditions and exposed to 12 h of daylight/12 h of night cycle. They were fed with normal rat chow and allowed water ad libitum. They were healthy rats and did not show signs of the presence of any infection neither had they been used for any other experiments.

The experimental protocol was performed according to the College of Medicine, King Faisal University Animal Care and ethics.

2.2. Collection of Sample

Rats were anaesthetized with urethane, positioned face down. A sterile cotton swab moistened in 0.9% saline solution was used for the collection of nasal samples. Swabs were collected by rubbing the sterile moist cotton-tipped swab in their nasal cavity and then inoculated into nutrient broth. The inoculated nutrient broth was incubated aerobically overnight at 37˚C. The obtained overnight growth was plated out on blood agar

and incubated aerobically for 24 hrs at a temperature of 37˚C. Pure cultures of the overnight growth were obtained by plating individual colonies on separate blood agar and incubated aerobically at 37˚C for 24 hrs.

2.3. Identification of Isolate and Antibiogram Test

All isolated pure colonies of obtained bacteria were identified using the Vitek 2 compact automated system (BioMerieux, Marcy L'Etoile, France) according to the manufacturers' guidelines. The GP card (AST-P586) was used to identify the Gram positive isolates and only the Staphylococci isolates were used for the investigation.

2.4. Determination of Minimum Inhibitory Concentration and Antibiotic Susceptibility

Minimum inhibitory concentrations (MIC) as well as the antibiotic susceptibility of isolates were also determined using the Vitek 2 compact automated system against the following antibiotics: Ampicillin/Sulbactam, Cefuroxime, Cefuroxime Axetil, Imipenem, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Quinpristin/Dalfopristin, Linezolid, Teicoplanin, Vancomycin and Tetracycline. The following antibiotics were not claimed by the Vitek machine: Benzlpenicillin, Ampicillin, Gentamicin High Level (synergy) and Streptomycin High Level (synergy). Sensitivity and resistance results were indicated by the Vitek 2 compact automated system.

2.5. Statistical Analysis

The obtained data was analysed using the 2012 SPSS Version 19. Chi square test was used to compare the relationship between sensitivity and resistance to antibiotics by the isolates. Significant association was set at $p < 0.05$.

3. Results

3.1. Staphylococcal Species Encountered

The results obtained showed that four different strains of *Staphylococcus aureus*, were encountered. They were labelled as *S. aureus* 1, 2, 3 and 4 respectively. Also encountered was *Staphylococcus lugdunensis* as well asother coagulase negative Staphylococci species.

3.2. Antibiotic Susceptibility

The isolates had been tested against sixteen antibiotics and the results of their susceptibility as well as their minimum inhibitory concentrations (MIC) are shown in Table 1 and Table 2.

The obtained results showed all (100%) of the *Staphylococcus aureus* isolates to be resistant to methicillin as is presented in Table 1. For *S. aureus* isolate 1, there was a 69% resistance exhibited by this isolate against the tested antibiotics. The isolate was also resistant to methicillin as well as to vancomycin. The difference between the numbers of antibiotics resistant and sensitive to by *S. aureus* isolate 1 is seen to be statisti cally significant with p-value 0.034 and the results are as shown in Table 3. Also seen to be resistant to methicillin and vancomycin is *S. aureus* isolate 4 which showed a 62%

Table 1. Antibiotic susceptibity of isolates to tested antibiotics.

ISOLATES	ANTIBIOTICS															
	AMX/SUL	CEF	CEF/Axe	IMP	LEVO	MOX	ERY	CLIN	QUI/DAL.	LINZ	TEI	VAN	TETR	TG	NIT	SXT
***S. aureus* 1**	R	R	R	R	S	S	R	R	R	R	R	R	R	S	S	S
***S. aureus* 2**	R	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S
***S. aureus* 3**	R	R	R	R	S	S	R	R	S	S	S	S	R	S	S	S
***S. aureus* 4**	R	R	R	R	S	S	R	R	R	S	R	R	R	S	S	S
S. lug.	R	R	R	R	S	S	I	S	S	S	S	S	S	S	S	S
OCNS	R	R	R	R	S	S	S	R	S	S	I	I	S	S	S	S

R = Resistant; I = Intermediate; S = Sensitive; *S. lug.* = *S. lugdunensis*; OCNS = Other Coagulase Negative Staphylococci; AMP = Ampicillin/Sulbactam; CEF = Cefuroxime; CEF/AXE = Cefuroxime/Axeti; IMP = Imipenem; LEVO = Levofloxacin; MOX = Moxifloxacin; ERY = Erythromycin; CLD = Clindamycin; QU/DA = Quinupristin/Dalfopristin; LIZ = Linezolid; TEI = Teicoplanin; VAN = Vancomycin; TET = Tetracycline; TG = Tigecycline; NIT = Nitrofurantoin; SXT = Trimethr/Sulfamethoxazole.

Table 2. Minimum inhibitory concentration of against tested antibiotics.

Isolates	Antibiotics															
	AMP/	CEF	CEF/Axe.	IMP.	LEVO.	MOX	ERY	CLIN.	QU/DA	LINZ	TEI	VAN	TETR	TG	NITR	SXT
***S. aureus* 1**	≤2	8	8	≤1	0.5	0.5	≤8	≤8	8	≥8	≥32	≥32	≥16	≤0.12	≤16	≤10
***S. aureus* 2**	≤2	≤1	≤1	≤1	0.25	≤0.25	≥8	≤0.25	1	2	≤0.5	≤0.5	≤1	≤0.12	≤16	≤10
***S. aureus* 3**	≤2	2	2	≤1	0.25	≤0.25	≥8	≤0.25	0.5	1	≤0.5	≤0.5	≥16	≤0.12	≤16	≤10
***S. aureus* 4**	≤2	8	8	≤1	0.5	≤0.25	≥8	≥8	4	4	≥32	≥32	8	≤0.12	≤16	≤10
S. lugdunensis	≤2	≥64	≥64	≤1	≤0.12	<−0.25	1	≤0.25	0.5	1	≤0.5	1	≤1	≤0.12	32	≤10
OCNS	≤2	≥64	≥64	≤1	1	≤0.25	0.5		≥8	1	≤0.5	8	≤1	≤0.12	≤16	≤10

AMP = Ampicillin/Sulbactam; CEF = Cefuroxime; CEF/AXE = Cefuroxime/Axeti; IMP = Imipenem; LEVO = Levofloxacin; MOX = Moxifloxacin; ERY = Erythromycin; CLD = Clindamycin; QU/DA = Quinupristin/Dalfopristin; LIZ = Linezolid; TEI = Teicoplanin; VAN = Vancomycin; TET = Tetracycline; TG = Tigecycline; NIT = Nitrofurantoin; SXT = Trimethr/Sulfamethoxazole.

Table 3. Comparison between resistance and sensitivity against the tested antibiotics by Staphylococci isolates.

Isolate	Total No. Antibiotics.	No. Resistant	No. Sensitive	Intermediate	p-Value
***Staphylococcus aureus* 1**	16	11 (68.75%)	5 (31.25%)	0	0.034*
***Staphylococcus aureus* 2**	16	6 (37.5%)	10 (62.5%)	0	0.15854
***Staphylococcus aureus* 3**	16	7 (43.75%)	9 (56.25%)	0	0.4777
***Staphylococcus aureus* 4**	16	10 (62.5%)	6 (37.5%)	0	0.15854
Staph. lugdunensis	16	4 (25%)	11 (68.75%)	1 (6.25%)	0.01046*
OCNS	16	5 (31.25%)	9 (56.25%)	2 (12.5%)	0.13104

*The result is significant at $p < 0.05$; OCNS = Other Coagulase Negative Staphylococcus.

resistance against the tested drugs. There was, however, no significant difference between the number of antibiotics to which this isolate was sensitive or resistant to.

In the case of *S. aureus* isolates 2 and 3, there was a 37.5% and 43.75% resistance against the tested antibiotics respectively. However, the results presented in Table 2 showed that the difference between resistance and sensitivity in these isolates were not statistically significant. A similar pattern to that of *S. aureus* isolates 2 and 3 was seen amongst other coagulase negative Staphylococcus which showed a 31.25% resistance against the tested antibiotics.

In the case of *Staphylococcus lugdunensis* of the tested antibiotics, the bacterium was resistant to 25% of the tested antibiotics and intermediate to 6.25% of the drugs. There was, however, a statistically significant difference between the number of antibiotics sensitive or resistant to and the results are shown in Table 2.

Generally, according to antibiotic groups, all (100%) the isolates were resistant to the Penicillins, Cepharlosporins and Imipenem. There was an 83% resistance to Lincosamides, while 67% of the isolates were resistant to Macrolides, half (50%) of them were resistant to tetracycline. For Vancomycin, Streptogramin and Teicoplanin, 33% of the isolates were each resistant to the antibiotics and the results are presented in Figure 1.

4. Discussion

The colonization of nasal snares by *Staphylococcus aureus* similar to those of humans is seen being exhibited by laboratory bred rats as shown in the present report. The result obtained in the present investigation further confirms the zoonotic carriage of Methicillin resistant Staphylococcal aureus (MRAS) infections in the nasal snares of laboratory bred rats. That some of these isolates are also vancomycin resistant further highlights the public health problem of bacterial species which are difficult to treat existing in animal reservoirs. It is also worth noting that the rats in the present investigation are laboratory bred and have never been in the field. There is no available history of them having at any time been treated with antibiotics. Therefore, it would imply that there has probably been a human to animal transfer of MRSA and VRSA. Transmission of MRSA between humans and animals has been reported by various researchers [11] [23]

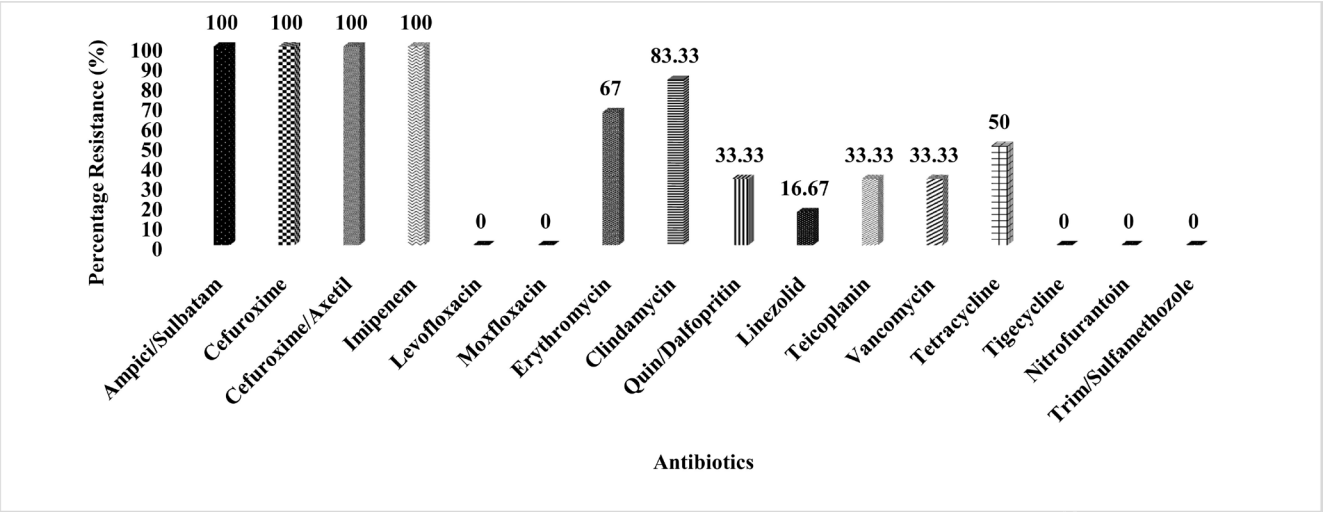

Figure 1. Percentage of resistance of Staphylococci isolates against the tested antibiotics.

[24]. However, while Urdahl *et al.* [24] stated that all MRSA variants could be transmitted between humans and animals with the bacteria rarely producing disease in humans and animals, they cautioned the spread to hospitals, health institutions and nursing homes. This might therefore, explain why the rats in this study were healthy and there were no any reported cases of MRSA infection incidences among the human handlers.

Four strains of *S. aureus* were isolated from the nasal snares of these laboratory bred rats in the present investigation. This is not unexpected as there have been reports of as many as 90 Staphylococci strains being isolated from human samples [2] with one of this strains being *S. lugdunensis*. The rats from which four strains of MRSA, two of which were also resistant to vancomycin had been co-habiting with *S. lugdunensis* as well as other CoNS. According to a report by Emerson [2] of the 187 hospital patients in the study, only 5.9% of them were found to have nasal carriages of *S. aureus* if there was also the carriage of *S. lugdunensis* while there was a higher (34.7%) *S. aureus* carriage in the absence of *S. lugdunensis*. This therefore, implies that co-habitation of *S. aureus* with *S. lugdunensis*, resulted in the inhibition of growth of *S. aureus* [2]. The results from the present investigation however, suggests a co-habitation of *S. aureus* and *S. lugdunensis* in which the presence of the later did not fend off the existence of MRSA or vancomycin resistant *S. aureus*. This association could be said to be similar to the 5.9% hospital patients in the report by Emerson [2] in which there was a reduced co-habitation with *S. aureus* and *S. lugdunensis*. It might be worth noting that the *S. lugdunensis* in the present report, though sensitive to most antibiotics to which they had been tested, was also resistant to penicillin. There might therefore, be the possibility that the encountered *S. lugdunensis* in the present report could be a mutant strain that might be a non lugdunin producer. Also, that all the Staphylococci strains in the present investigation were resistant to the Penicillins with some being resistant to vancomycin does suggest that the presence of *S. lugdunensis* did not help in preventing resistance to the antibiotics amongst the other Staphylococci isolates. Neither did the nasal colonization with *S. lugdunensis* hinder the growth of MRSA, VRSA, nor the presence of other coagulase negative Staphylococci. There might be a need for further investigations on both human and animal co-habitations of *S. lugdunensis* with *S. aureus*, information that could help in the prevention of bacteria resistance to lugdunin in the future.

5. Conclusion

In conclusion, this study has revealed co-habitation of *Staphylococcus lugdunensis* with MRSA, VRSA, *S. aureus* as well as with other coagulase negative Staphylococci in laboratory bred rats. As these rats have existed only in the laboratory, these *S. aureus*, MRSA and VRSA isolates might be human strains. Also, that the presence of *S. lugdunensis* did not prohibit this co-habitation in the nasal snares of the rats, might suggest the possibility of "a non-lugdunin" producing *S. lugdunensis* strain. As pharmaceuticals look into the possibility of the new antibiotic "lugdunin", there might be the need to look into the possibility of mutant strains of this bacterium found not only in humans but also in human companions to curtail the problem of resistance to lugdunin.

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Conflict of Interest

The author declares none. The research was not funded.

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