Characterization and antibiotic resistance of *S. aureus* isolated from rabbits (*Oryctolagus cuniculus*)

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AIMS OF THE STUDY

An intra-farm study was carried out to characterize: -phenotypically and genotypically the *Staphylococcus aureus* (*SA*) strains isolated from skin and lesion swabs of different rabbit categories and farm workers; -the predominant *SA* clonal lineages; -the antibiotic resistance profiles; -the risk of zoonotic infection.

INTRODUCTION

The epidemiology of *SA* infections has gained interest in the last years for their importance in veterinary medicine, the emergence of some clonal animal lineages, and their increasingly evidenced zoonotic potential. *SA* infections represent a major problem in intensive breeding rabbit farms[1] (Fig. 1), because of the discovery of emergent *mec*C MRSA, and in companion rabbits[2] for the sporadic reports of MRSA[3-7]. Moreover, livestock-associated clonal complex ST398 MRSA[3] and the Panton-Valentine Leucocidin-positive isolates[5] have raised concern about the *SA* population in rabbits in which it causes pododermatitis lesions, abscesses and mastitis responsible of production problems, infertility and death[8] (Fig. 2, 3).

MATERIALS AND METHODS

Fig. 1. Intensive breeding farm of rabbits.

- Intensive breeding farm of rabbits for meat production in Lazio Region, Italy;
- average production: 30.000 rabbits/year;
- mortality: 8-9% nest; 7-8% fattening;
- semestral RHD virus vaccination;
- reproducers: 70% internal, 30% external;
- replacement 90% brood mare/year;
- age of weaning: 35 days; age of slaughter: 82-90 days;
- antibiotics: doxycycline, zinc bacitracin, valnemulin.

Fig. 2. Periocular blepharitis

Sampling

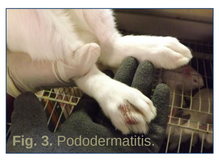
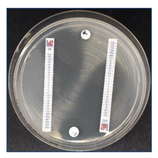


Fig. 3. Pododermatitis.

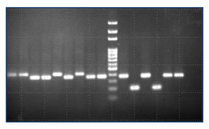
- 2200 swabs (ear, nose, armpit, groin, perineum, skin abscesses) from 400 rabbits, randomly selected belonging to different categories (youngs, adults, reproducers and new reproducers);
- 16 nasal and hand skin swabs from farm workers.

Phenotypic analysis



- Isolation and identification of *SA* by selective culture media and biochemical tests;
- antibiotic resistance profiles by Kirby Bauer and E-test method (Eucast 2017);
- MRSA identification (oxacillin and cefoxitin: Eucast 2017 recommendations).

Genotypic analysis



On a random selected number of animal and human strains (n=98) research of:

- *nuc* gene, *mec*A, *mec*C, *bbp*, *selm, flank* virulence genes[9,10]
- *spa*-type[9] (software Ridom Staph Type) clustering in CCs (algoritm BURP-Based Upon Repeat Pattern).

The statistical analysis was performed by Software STATA version 13.0

RESULTS

On 3376 isolate, 592 *SA* were detected (frequency of 17%). A significant difference in *SA* prevalence was recorded in relation to the age ranges ($P<0.05$), except for young and reproducers ($P=0.785$) (Fig. 4). All areas resulted infected, recording a significant difference between each skin area and lesions ($P<0.05$), while relevant *SA* prevalence was detected both in the ear (38%, n=452) and the nasal cavity (41%, n=395; $P=0.925$) (Fig. 5).

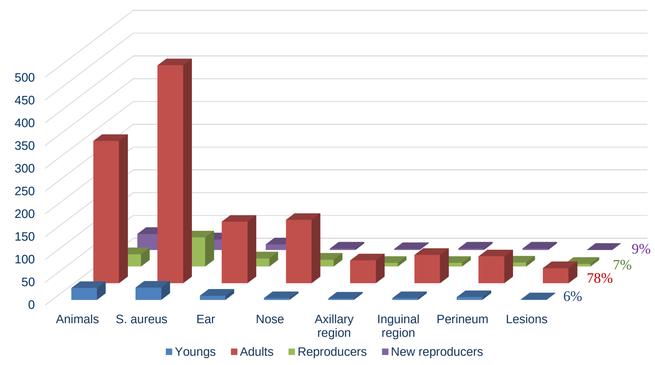


Fig. 4. Animal categories (N, %) and *SA* distribution (N) in relation to the age ranges and the sampled areas.

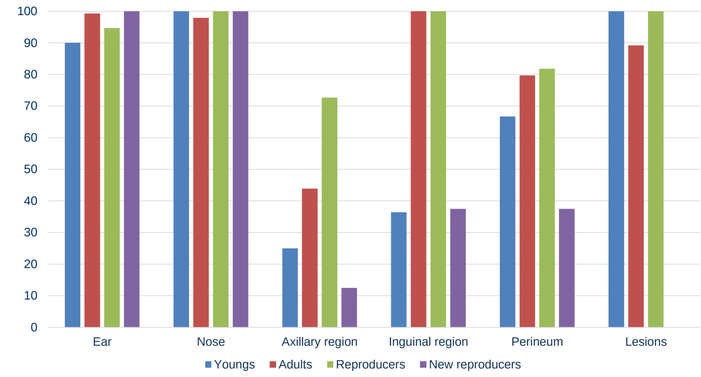


Fig. 5. *SA* prevalence (%) compared to total bacteria isolated by age ranges and skin sampled areas.

- All strains resulted MSSA but showed some multi-resistance profiles, ranging from 3 to 7 antibiotic classes: in particular tetracyclines (96%), macrolides (94%), diterpenes (84%), fluoroquinolones (64%), aminoglycosides B-C grades (48%-5%), and glycopepetides (teicoplanin: 73%; hVISA: 4%; VISA: 5%) (Fig. 6).

- All strains were negative for the presence of virulence genes and therefore were classified as low virulence strains. Five different *spa*-types were identified, belonging to two different clonal complexes (CC97, CC15). The most frequently recovered *spa*-type has been t2802 (55%), also detected in human samples (Table 1, Fig. 7).

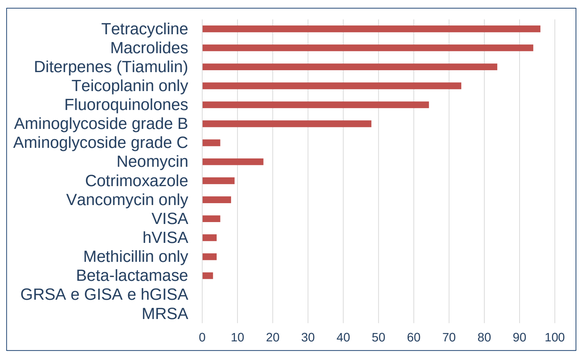


Fig. 6. Antibiotic resistance profiles of *SA* strains.

Table 1. CC-*spa* and *spa*-type analysis results.

# Isolates	CC-spa	*spa*-type	repeat succession	ass. CCs	VF	Isolate	Resistance profile
54 (55.1%)	267	t2802	07-23-21-17-34-34-34-33-34	97	-	MSSA	SP-NOR-TE-CN-E-CD-TOB
36 (36.7%)	084	t491	26-23-12-34-34-12-12-23-02-12-23	15	-	MSSA	SP-TE-E-CD
4 (4.1%)	084	t094	07-23-12-34-34-12-12-23	15	-	MSSA	P-NOR-TE
2 (2.0%)	012	t2036	26-23-12-34-34-12-23-02-12-23	15	-	MSSA	SP-TE-E-CD
2 (2.0%)	Excluded	t605	07-23	-	-	MSSA	SP-TE-CN-N-E-CD-TOB

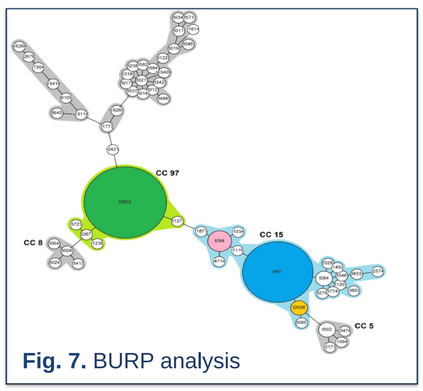


Fig. 7. BURP analysis

CONCLUSIONS

In this study a high frequency of multiresistant *SA* strains was observed in rabbits, although none could be classified as MRSA. Clonal lineages were not correlated to the sampling site, while an association was recorded with the antibiotic-resistance profiles.

REFERENCES

1- Agnoletti *et al.* 2014. *Vet Microbiol* 170: 172-177. 2- Holmes *et al*. 2016. *PLoS ONE* 11(3): e0151458. 3- Loncaric and Künzel 2013. *Vet Dermatol* 24(3), e84: 370-372. 4- Rich and Roberts 2006. *Vet Rec* 159(16): 535-536. 5- Rankin *et al*. 2005. *Vet Microbiol* 108(1-2): 145-148. 6- Hermans *et al*. 2003. *Vet Microbiol* 67: 37-46. 7- Ortega *et al*. 2009. *Rev Sci Tech* 28: 1119-1128. 8- Meulemans *et al*. 2007. *Vet Microbiol* 125: 333-340. 9- DTU food, 2009 http://www.crl-ar.eu/data/images/tc_april-2009/who%20meca-pcr_protocol%209-06-11.pdf., 10- Vancraeynest *et al*., 2007. *Vet Microbiol* 121: 368-372.