PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

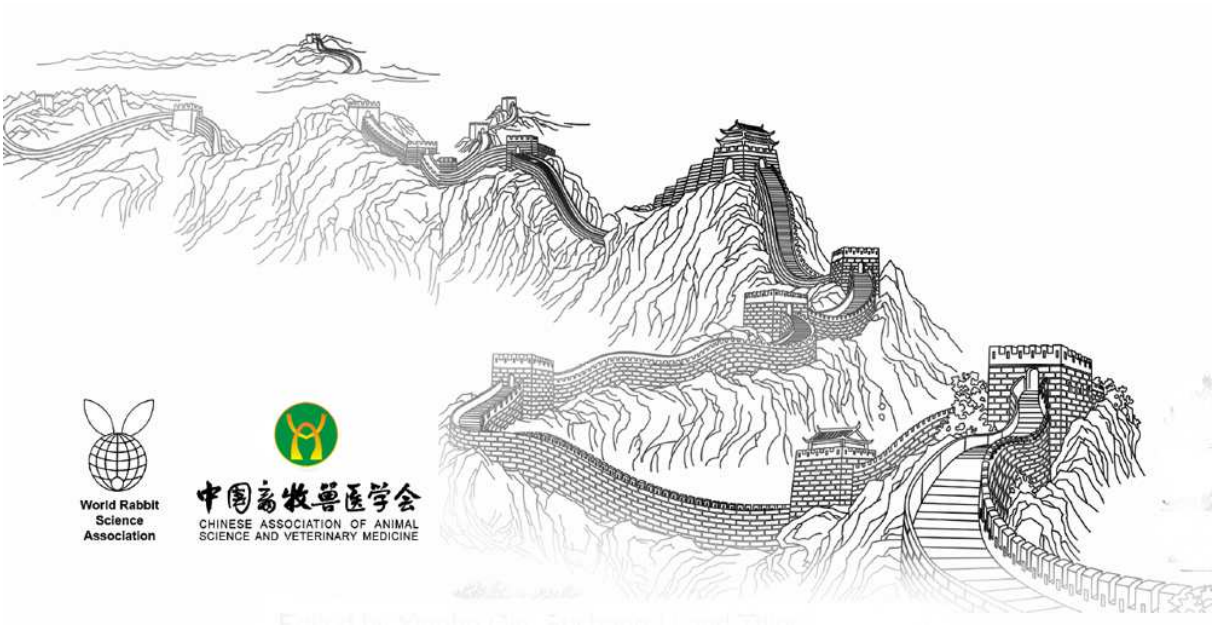
Session
Pathology and Hygiene

Brunetta R., Mazzolini E., Bano L., Berto G., Guolo A., Ferro T., Puiatti C., Rigoli R., Tonon E., Zandonà L., Drigo I., Agnoletti F.

A THREE-YEAR PROSPECTIVE STUDY SHOWS CLONAL SPREADING OF T5210 ST398 MRSA IN RABBITS AND FARM WORKERS OF ONE INDUSTRIAL FARM.

Full text of the communication

How to cite this paper :
Brunetta R., Mazzolini E., Bano L., Berto G., Guolo A., Ferro T., Puiatti C., Rigoli R., Tonon E., Zandonà L., Drigo I., Agnoletti F., 2016 - A three-year prospective study shows clonal spreading of t5210 ST398 MRSA in rabbits and farm workers of one industrial farm.. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 517-520.*



A THREE-YEAR PROSPECTIVE STUDY SHOWS CLONAL SPREADING OF t5210 ST398 MRSA IN RABBITS AND FARM WORKERS OF ONE INDUSTRIAL FARM

Brunetta R.[1], Mazzolini E.[1], Bano L.[1], Berto G.[1], Guolo A.[1], Ferro T.[1], Puiatti C.[1], Rigoli R.[2], Tonon E.[1], Zandonà L.[1], Drigo I.[1], Agnoletti F.[1]

[1] Istituto Zooprofilattico Sperimentale delle Venezie, via dell'Università 10, 35020 Legnaro (PD), Italy
[2] Dipartimento di Patologia Clinica, Ospedale Santa Maria di Cà Foncello, 31100 Treviso, Italy
[*]Corresponding author: fagnoletti @izsvenezie.it

ABSTRACT

In 2013 we reported ST398 methicillin-resistant *Staphylococcus aureus* (MRSA) first detection in rabbits of an industrial holding. Since, we followed up this farm for three years (four sample collections) to study the within-herd epidemiology and the exposure to MRSA of farm workers and their families. The sampling of does was carried out by systematic selection of cages. All *Staphylococcus aureus* (*S. aureus*) isolates from rabbit and human specimens detected after pre-enrichment in broth and culture onto selective agar were tested by PCR for *mecA* and *mecC*. All the human MRSA isolates and a selection of rabbit MRSA isolates were further characterized by *spa* typing and MLST typing. The percentage of rabbits carrying *S. aureus* ranged from 53% to 93% of tested rabbits in four samplings. Out of the *S. aureus* isolates, MRSA was 52% at beginning and 25% after five months, but raised to 100% after one year and up to the third year of follow up. Initially t034 was the prevalent *spa* type among MRSA, but t5210 become by far the most frequent one after one year (16/23 isolates) and three years (25/28 isolates), when t034 was seldom found among MRSA isolates. Both t034 and t5210 belonged to sequence type ST398. Overall during the study 16 persons volunteered to participate. At first and second sampling t034 and t5210 were found in two and four farm workers, respectively, but later on t034 was never detected in farm workers and t5210 was instead found contaminating all of them. Overall ten farm workers' relatives provided samples, five were found carrying MRSA, four of them were t5210 MRSA carriages. We believe the rabbit intensive breading system may be among the herds that increase the burden of exposure of person to LA-MRSA, however further studies are needed to understand whether the rabbit production system is capable to sustain the spreading of LA-MRSA.

Key words: methicillin-resistant *Staphylococcus aureus* (MRSA), ST398, livestock associated MRSA (LA-MRSA), rabbit, zoonoses

INTRODUCTION

Staphylococcus aureus (*S. aureus*) methicillin resistant (MRSA) is among the multi-drug resistant microorganisms causing hospital or community-acquired infections that are currently challenging healthcare worldwide (WHO, 2014). Since 2003 (Voss et al., 2005), MRSA clones have been over and over reported from different animal species, including domestic animals reared for companion or for food production. In animals, MRSA is mostly carried with no apparent disease, yet animal carriage exposes humans, primarily farm workers, veterinarians or slaughterhouse workers, to contamination by direct contact or airborne transmission. Indeed, it has been previously evidenced that, unless swine farm workers of MRSA positive holdings wear protective facemasks they are at high risk to become contaminated (van Cleef et al., 2015). The clonal complex CC398 of MRSA, especially the sequence type ST398, is mostly represented among LA-MRSA being reported in swine, dairy, veal calf, poultry and companion animals. Despite *S. aureus* is among the most relevant bacteria affecting rabbits, MRSA was initially found only in pet rabbits and attributed to exposure to humans (Loncaric and Kunzel, 2013). In 2013 we found LA-MRSA in an industrial meat rabbit holding and in the nostrils of farm workers or their family members (Agnoletti et al., 2014). Since, we followed up this farm to

study the within-herd epidemiology and the exposure of farm workers and their families to MRSA by this food-producing animal. This presentation reports results from the three-year follow up.

MATERIALS AND METHODS

Once an industrial holding that bred rabbits for meat was found contaminated with ST398 MRSA in 2013 (Agnoletti et al., 2014), we followed it up by sampling 60 does after systematic selection of cages at 5th, 12th and 33th month. Farm workers and their family members, who provided informed consensus and volunteered to participate, were also sampled at the same time to detect MRSA in their nostrils. Rabbit specimen collection and the microbiologic procedure to isolate and type *S. aureus* were performed as previously described (Agnoletti et al., 2014). Briefly, *S. aureus* isolation from rabbit specimens was performed by pre-enrichment in broth (Heart Infusion Broth, Oxoid) and culture onto selective agar (Baird-Parker Agar Base and RPF Supplement, Oxoid). The human nasal swabs were processed on enrichment and selective media for MRSA isolation (Tryptone Soy Broth supplemented with cefoxitin (3.5 mg/L) and aztreonam (75 mg/L), and CHROMagar MRSA II, BBL). All *S. aureus* colonies were tested for *mecA* (Louie et al., 2002) and *mecC* (Paterson et al., 2012). *S. aureus* identification was performed by mass spectrometry (Maldi Biotyper, Bruker Daltonics) and *nuc* detection by PCR (Louie, et al., 2002). All the MRSA isolated from humans and a selection of MRSA isolated from rabbits were further characterized by *spa* typing (Shopsin et al., 1999) and multi locus sequence typing (MLST) (Enright et al., 2000). To classify and identify *spa* types the sequences were analyzed with the Ridom StaphType software program (version 1.4; Ridom, GmbH,Wurzburg, Germany [http://spa.ridom.de/index.shtml]). Sequence type (ST) was assigned based on the sequence allelic profiles using the MLST database website (http://www.mlst.net).

RESULTS

Methicillin-resistant *S. aureus* in rabbits and humans was always found over the three-year prospective study. Results of testing and typing *S. aureus* isolates from rabbits and humans are shown in table 1 and table 2. The percentage of rabbits contaminated with *S. aureus* ranged from 53% to 93% of tested animals over the three-year period. As we previously reported, the proportion of MRSA among rabbit that were carrying *S. aureus* were 52% and 25% at first and second sampling, respectively (Agnoletti et al., 2014); in the follow up of this case report, after 12 and 33 months from first detection, all (100%) *S. aureus* isolates from sampled rabbits displayed resistance to methicillin.

Table 1: Results of four samplings over three years in one MRSA-contaminated industrial rabbit holding: for each sampling *S. aureus*- and of MRSA-positive rabbits numbers are reported, together with the *spa* types and the MLST types.

Sampling month	Rabbits tested (N.)	Rabbits *S. aureus* carriage (%)	MRSA carrier rabbits (N.)	MRSA rabbit carrier among *S. aureus* carriage	MRSA molecular typing results				
					MRSA isolates typed	N.	*Spa* type	MLST type (ST)	LA-MRSA (%)
0	25	23 (92%)	12	52%	3	1	t5210	398	66%
						1	t034	398	
						1	t121	159	
5th	60	59 (98%)	15	25%	15	7	t034	398	66%
						3	t5210	398	
						2	t1190	Not found	
						2	t2970	Not found	
						1	t159	121	
12th	60	32 (53%)	32	100%	23	16	t5210	398	100%
						7	t13617	398	
33rd	60	56 (93%)	56	100%	28	25	t5210	398	100%
						1	t011	398	
						1	t034	398	
						1	t15492	398	

Initially, t034 was the prevalent *spa* type among MRSA, but t5210 become by far the most frequent one after one year (16/23 isolates) and three years (25/28 isolates), when t034 was seldom found among MRSA isolates. Both t034 and t5210 belonged to MLST ST398. Other two *spa* types, also typed ST398, were found in rabbits at 3rd and 4th sampling: t13617 (seven isolates) and t15492 (one isolate), respectively (table 1).

Overall, during the study 16 persons volunteered to participate. Sampling was discontinuous for family members, whereas farm workers were sampled almost regularly during the prospective study. MRSA was detected in all six farm workers. At first and second sampling, t034 and t5210 were found in two and four farm workers as already reported (Agnoletti et al 2014). At 3rd and 4th sampling, t034 was never detected in farm workers, t5210 was instead found contaminating all of them. Overall, out of the ten farm workers' relatives who provided samples, five were found carrying MRSA and four of them were t5210 carriers. One relative was found t034 contaminated for two consecutive samplings (2nd and 3rd). Four other relatives were sampled once at the last sampling and they were not found contaminated with MRSA (table 2).

Table 2: Results of testing MRSA contamination of nostrils of farm workers and their relatives exposed in one LA-MRSA contaminated industrial holding. Humans were sampled four times over three years. MRSA human isolates were typed by *spa* typing and multi locus sequence typing.

Person's ID	Person role within the study	MRSA *spa* type	Sampling month			
			0	5th	12th	33rd
1	farm worker	5210	1	1	nt	1
2	farm worker	34	1	nt		
		5210		nt		1
		13617		nt	1	
3	farm worker	5210	1	1	1	1
4	farm worker	5210	nt	1	1	1
5	farm worker	34	1			
		5210		1	1	
6	farm worker	5210			1	
7	relative	5210	1	nt	nt	nt
8	relative		nt	0	nt	0
9	relative	5210	nt	1	nt	
10	relative	5210	nt	0	1	
11	relative	5210	nt	nt	nt	1
12	relative	34	nt	1	1	
13	relative		nt	nt	nt	0
14	relative		nt	nt	nt	0
15	relative		nt	nt	nt	0
16	relative		nt	nt	nt	0

DISCUSSION

Over the prospective study LA-MRSA (ST398) was always detected in animals and humans. It was also found contaminating the building air and surfaces (Agnoletti et al., 2014). Proportions of MRSA carrier rabbits increased over time and all *S. aureus* carrier rabbits were actually MRSA carriages after one year and up to the third year we followed this holding. Results show that MRSA substituted MSSA within this herd. Furthermore the clone t034, we detected at beginning, was substituted with clone t5210. Intra-herd prevalence in LA-MRSA positive holdings is directly related to transfer of LA-MRSA to humans (Graveland et al., 2010), indeed we found the same dynamics of increasing proportions of LA-MRSA carriers and dissemination of a single *spa* type MRSA clone in people exposed directly or indirectly to rabbits, t5210 was the clone colonising the nostrils of all farm workers and some of their relatives. Though t034 was found in one relative, we believe this person was exposed to other livestock contamination. In this holding, rabbits and humans seem to share the same adaptation of MRSA to intensive breeding. In simulation studies one LA-MRSA positive pig farm may be sufficient to initiate an epidemic in humans, provided sufficient amounts of persistently contaminated animals and humans carriers are available (Porphyre et al., 2012). The

rabbit intensive system may be considered among the herds that increase the burden of exposure of humans to LA-MRSA, however further studies are needed to understand whether the rabbit production system is capable to sustain the spreading of LA-MRSA. In the here described holding, peculiar condition of heavy exposure to other livestock may have acted to trig the internal dissemination of LA-MRSA.

CONCLUSIONS

In conclusion, after a prolonged follow up of a rabbit industrial holding that was found contaminated with LA-MRSA, we had proof of within-herd spreading of clone t5210 MRSA that was able to displace MSSA and other MRSA *spa* types. This clone has high capability to contaminate humans directly exposed, by the holding environment and animals, and indirectly by means of family connections. Despite LA-MRSA has been found and reported only in one holding of the meat rabbit breeding sector, the high within-herd spreading ability of this clone should be accounted for biosecurity plans.

REFERENCES

Agnoletti F., Mazzolini E., Bacchin C., Bano L., Berto, G. Rigoli R., Muffato G., Coato P., Tonon E., Drigo I., 2014. First reporting of methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in an industrial rabbit holding and in farm-related people. *Vet. Microbiol., 170, 172-177.*

Enright M.C., Day N.P., Davies C.E., Peacock S.J., Spratt B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J. Clin. Microbiol., 38, 1008-1015.*

Graveland H., Wagenaar J.A., Heesterbeek H., Mevius D., van Duijkeren E., Heederik D., 2010. Methicillin resistant Staphylococcus aureus ST398 in veal calf farming: Human MRSA carriage related with animal antimicrobial usage and farm hygiene. *PLoS One, 5, e10990.*

Loncaric I., Kunzel F., 2013. Sequence type 398 methicillin-resistant Staphylococcus aureus infection in a pet rabbit. *Vet. Dermatol., 24, 370-e84.*

Louie L., Goodfellow J., Mathieu P., Glatt A., Louie M., Simor A.E., 2002. Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. *J. Clin. Microbiol., 40, 2786-2790.*

Paterson G.K., Larsen J., Harrison E.M., Larsen A.R., Morgan F.J., Peacock S.J., Parkhill J., Zadoks R.N., Holmes M.A., 2012. First detection of livestock-associated meticillin-resistant *Staphylococcus aureus* CC398 in bulk tank milk in the United Kingdom. *Euro Surveill., 17, 20337.*

Porphyre T., Giotis E.S., Lloyd D.H., Stark K.D., 2012. A metapopulation model to assess the capacity of spread of methicillin-resistant Staphylococcus aureus ST398 in humans. *PLoS One, 7, e47504.*

Shopsin B., GomezM., MontgomeryS.O., Smith D.H., Waddington M., Dodge D.E., Bost D.A., Riehman M., Naidich S., Kreiswirth B.N., 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J. Clin. Microbiol., 37, 3556-3563.*

van Cleef B.A., van Benthem B.H., Verkade E.J., van Rijen M.M., Kluytmans-van den Bergh M.F., Graveland H., Bosch T., Verstappen K.M., Wagenaar J.A., Bos M.E., Heederik D., Kluytmans J.A., 2015. Livestock-associated MRSA in household members of pig farmers: Transmission and dynamics of carriage, a prospective cohort study. *PLoS One 10, e0127190.*

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant staphylococcus aureus in pig farming. Emerg. Infect. Dis. 11, 1965-196

World Health Organization (WHO), 2014. Antimicrobial resistance global report on surveillance. *http://Apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua.*

==========