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Research note

Clinical impact and prevalence of MRSA CC398 and differences between MRSA-Tet^{R} and MRSA-Tet^{S} in an area of Spain with a high density of pig farming: a prospective cohort study☆

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ABSTRACT

Objectives: Tetracycline resistance (Tet^{R}) is a phenotypic marker of the livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 clone. The aim of this study was to analyse the prevalence of MRSA CC398 in patients in contact with healthcare facilities and differences between patients with MRSA-Tet^{R} and MRSA tetracycline-susceptible (Tet^{S}) strains.

Methods: Patients diagnosed with MRSA from January 2012 to December 2015 were divided into two groups, MRSA-Tet^{R} and MRSA-Tet^{S}. Epidemiologic and clinical data were evaluated. Molecular analysis was performed (multilocus sequence typing, *spa* typing) on MRSA-Tet^{R} strains.

Results: Data from 288 MRSA patients were obtained, and 106 (36.8%) carried MRSA-Tet^{R} (93 typed as CC398 (87.7%); the remaining 13 isolates were ascribed to CC9, CC1, CC121, CC30, CC97, CC146 and CC152). The most frequent *spa* type was t011 (56.6%, 61/106). Detection of MRSA-Tet^{R} increased over the years (21.9%, 16/73, in 2012; 50.7%, 36/71, in 2015; p <0.001). Hospital acquisition was found in 16.7% (19/114) of MRSA-Tet^{R} patients vs. 83.3% (95/114) in MRSA-Tet^{S} patients (p <0.001). Frequency of MRSA-Tet^{R} patients in nursing homes was lower than in MRSA-Tet^{S} patients (4.7%, 5/106, vs. 27.5%, 50/182, p <0.001). MRSA-Tet^{R} as distinct from MRSA-Tet^{S} was associated with workers on pig farms (49.0%, 52/106, vs. 1.0%, 2/182; p <0.001), fewer admissions to hospital (46.2%, 49/106, vs. 68.1%, 124/182; p <0.001) and fewer comorbidities (81.1%, 86/106, vs. 59.9%, 109/182; p <0.001). Sixty cases of MRSA-CC398 infection were diagnosed, including, among others, endocarditis, septic arthritis, prosthetic joint infection, pneumonia and bacteraemia.

Conclusions: Prevalence of MRSA-Tet^{R} (especially CC398) at the hospital level in a Spanish region with intensive pig farming activity is high and is responsible for severe infections. Significant differences were detected in clinical and epidemiologic characteristics among MRSA-Tet^{R} and MRSA-Tet^{S} patients.

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Introduction

Livestock-associated (LA) methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified in animals and derived food as well as in humans in areas with extensive livestock production in Europe, North America and Asia; recently in sporadic cases in Africa

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have been reported [1,2]. The clonal complex CC398 is especially associated with pig farming. This complex is the main lineage in LA-MRSA, although other LA-MRSA clones have also been reported, such as CC9 and CC97 [3]. Previous studies have established that tetracycline resistance (Tet^R) is a good marker for rapid identification of CC398 strains among MRSA clinical isolates [4,5].

The county of Osona (Barcelona, Catalonia, Spain) is an area with a high density of pig farms. Furthermore, a high nasal carriage rate of MRSA ST 398 (all Tet^R) has been recently detected in pig farm workers and farm animals in the Osona region [6].

The objectives of this study were to analyse, in a hospital of the same geographic region, the clinical, epidemiologic and microbiologic differences between MRSA-Tet^R patients (especially of CC398 lineage) and MRSA-Tet^S patients; and the prevalence of infection and colonization by MRSA-Tet^R CC398 in patients in contact with healthcare facilities.

Material and Methods

A prospective study was carried out in the Hospital Universitari de Vic in the county of Osona (Barcelona, Spain) from January 2012 until December 2015.

Patient selection

All new patients with positive MRSA culture tests (for both infection and colonization) obtained between January 2012 and December 2015 were included in the study. Patients who were identified as carriers or as infected with MRSA in previous years were not included. Patients were divided into two groups depending on their tetracycline resistance phenotype (Tet^R and Tet^S). Sociodemographic and clinical data were studied. The data are provided in the Supplementary Material and Methods.

MRSA isolation and characterization

Detailed microbiologic methods can be found in the Supplementary Appendix.

Molecular typing

All MRSA strains were analysed by multilocus sequence typing (MLST) (http://saureus.mlst.net/) and were also characterized by *spa* typing [7]. The *spa* types were assigned according to the Ridom web server (http://www.spaserver.ridom.de) using RidomStaphType 1.4 software (Ridom, Münster, Germany).

The index of diversity (IOD) is the average probability that a typing system will assign a different type to two unrelated strains randomly sampled in the microbial population. The modified Hunter and Gaston IOD was calculated using the V-dice application among unrelated isolates (BioNumerics, http://www.hpa-bioinformatics.org.uk/cgi-bin/DICI/DICI.pl).

Minimum spanning trees were created by goeBURST [8] implemented in PHYLOWiz [9]. MLST were represented by circles; the size of a circle indicates the number of isolates of this particular sequence type (ST).

Statistical analysis

For quantitative variables, the mean and the standard deviation were described, and for qualitative variables, absolute frequencies and percentages were calculated. The chi-square test (Fisher's exact test) or chi-square test for trends and the Student's *t* test were used to compare clinical characteristics between patients in whom MRSA-Tet^R or MRSA-Tet^S was found.

In order to identify factors associated with MRSA-Tet^R, univariable logistic regression was performed, and subsequently, with the univariable factors associated with MRSA-Tet^R, multivariate logistic regression was carried out. Statistical significance was considered to exist when the p value was <0.05. Statistics 23.0 software (IBM SPSS, Chicago, IL, USA) was used for the statistical analyses.

Results

Of the 288 patients in whom MRSA was detected, 106 cases (36.8%, 95% confidence interval (CI) 31.24–42.38) were identified as being MRSA-Tet^R. The prevalence of MRSA-Tet^R increased over the study period (Fig. 1), going from 21.9% in 2012 to 50.7% in 2015 ($p < 0.001$). The prevalence of MRSA-Tet^R of lineage CC398 increased during the 4-year period, from 19.2% in 2012 to 45.1% in 2015 ($p < 0.001$) (Fig. 1).

MRSA-Tet^R typing

MLST was performed for the 106 MRSA-Tet^R strains, and 13 different STs were detected (IOD 0.308, 95% CI 0.192–0.42), comprising nine clonal complexes (CC) (Supplementary Table S1). The most frequent clonal complex was CC398 (87.7%. 95% CI 81.49–93.98), which included six different STs, with ST398 being the most representative, detected in 88 strains (83.0%, 95% CI 75.87–90.17). A total of 20 *spa* types were identified (IOD 0.65, 95% CI 0.550–0.750), with t011 accounting for most of the isolates ($n = 61$). The ST398/t011 combination was predominant ($n = 59$), and ST398 was associated with ten different *spa* types (Supplementary Fig. S1).

Colonization and infection

Over the 4-year period of the study, 169 patients (58.7%) were diagnosed with an MRSA infection, and MRSA-Tet^R was identified in 70 of them (41.4%). Sixty cases of MRSA-CC398 infections were diagnosed, including the following severe infections: cellulitis ($n = 28$), urinary tract infection ($n = 8$), cutaneous abscess ($n = 7$), pneumonia ($n = 5$), furunculosis, otitis and septic arthritis ($n = 2$ each), endocarditis, balanitis, bacteraemia, catheter infection, prosthetic joint infection and vaginitis ($n = 1$ each). Supplementary Table S2 shows the infections associated with MRSA-Tet^S and MRSA-Tet^R strains.

Differences between MRSA-Tet^R vs. MRSA-Tet^S and the risk factors associated with MRSA-Tet^R

The clinical and epidemiologic differences between the patients with MRSA-Tet^R and MRSA-Tet^S strains, as well as the risk factors associated with MRSA-Tet^R, are shown in Table 1.

A comparison of the rates of resistance for non-β-lactam antibiotics between the strains of MRSA-Tet^R and MRSA-Tet^S groups is provided in Supplementary Fig. S2. The resistance phenotypes of the MRSA-Tet^R strains are presented in Supplementary Table S1.

Discussion

Our study found that the prevalence of MRSA-Tet^R clinical isolates in an area with high pig density, like the county of Osona (Barcelona), at the expense mainly of the CC398 lineage (87.7%), is increasing, with significant progression during the 4-year period (more than 50% of the total of MRSA recovered strains in 2015).

Severe MRSA-Tet^R infections, particularly those caused by MRSA CC398 strains, were diagnosed in this period, such as endocarditis,

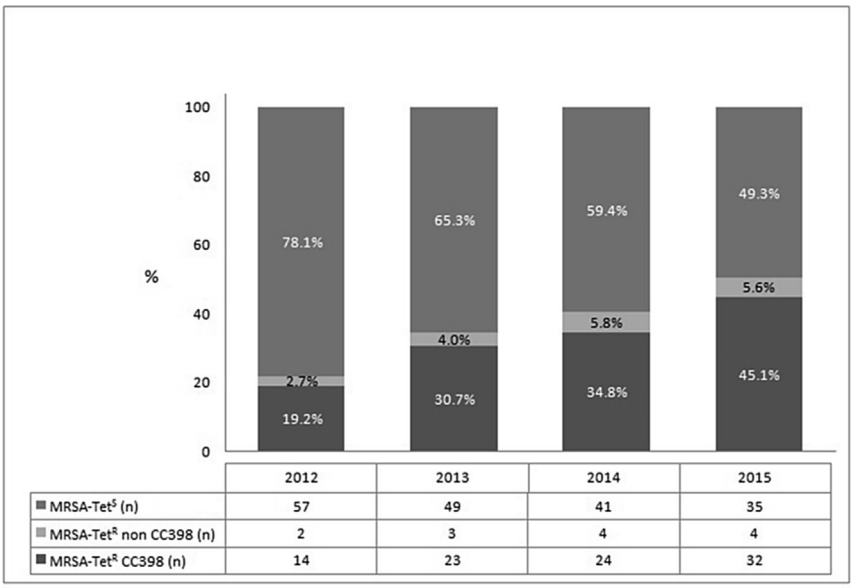

Fig. 1. Prevalence of MRSA-Tet^R (CC398 and non-CC398) and MRSA-Tet^S during the 4-year study period. MRSA, methicillin-resistant *Staphylococcus aureus;* Tet^R, tetracycline resistant; Tet^S, tetracycline susceptible.

Table 1
Univariate and multivariate analyses of epidemiologic differences and risk factors

Characteristic	MRSA-Tet^R ($n = 106$)	MRSA-Tet^S ($n = 182$)	Crude OR (95% CI)	p	Adjusted OR (95% CI)
Year					
2012	16 (21.9)	57 (78.1)	1	<0.001	1
2013	26 (34.7)	49 (65.3)	1.89 (0.91–3.93)		0.546 (0.16–1.85)
2014	28 (40.6)	41 (59.4)	2.43 (1.17–5.07)		3.32 (1.18–9.28)
2015	36 (50.7)	35 (49.3)	3.66 (1.77–7.56)		3.23 (1.14–9.15)
Age, years, mean ± SD	65.1 ± 22.7	73.6 ± 19.4	0.98 (0.97–0.99)	0.001	
Male gender	76 (71.7)	108 (59.3)	1.74 (1.04–2.91)	0.035	
MRSA acquisition					
Hospital acquired	19 (17.9)	95 (52.2)	1	<0.001	
Community acquired	87 (82.1)	87 (47.8)	5.00 (2.81–8.89)		
Living in nursing home	5 (4.7)	50 (27.5)	0.13 (0.05–0.34)	<0.001	
Mortality					
At 6 months	21 (19.8)	73 (40.3)	0.37 (0.21–0.64)	<0.001	
At <30 days	8 (40)	24 (32.4)	1.39 (0.50–3.85)	0.526	
Pig farmer	52 (49.0)	2 (1.0)	87.81 (20.69–372.55)	<0.001	288.79 (30.3–2750.7)
Hospital admission in previous 12 months	49 (46.2)	124 (68.1)	0.40 (0.25–0.66)	<0.001	
Barthel index, mean ± SD	86.4 ± 32.0	64.0 ± 23.7	1.03 (1.01–1.04)	<0.001	
Barthel index categorized					
0–20	6 (5.7)	29 (16.0)		<0.001	
21–60	7 (6.6)	58 (32.0)			
61–90	28 (26.4)	38 (21.0)			
91–99	0 (0)	2 (1.1)			
100	65 (61.3)	54 (29.8)			
Charlson index, mean ± SD[a]	4.43 ± 3.7	6.21 ± 3.4	0.87 (0.81–0.93)	<0.001	
Antibiotics[b]	72 (67.9)	159 (87.4)	0.31 (0.17–0.56)	<0.001	
Comorbidities[c]					
>4 comorbidities	20 (18.9)	73 (40.1)	1	<0.001	
<4 comorbidities	86 (81.1)	109 (59.9)	0.35 (0.20–0.62)		
Survival at 10 years, mean ± SD	41.4% ± 39.0%	20.9% ± 33.9%	1.02 (1.01–1.02)	<0.001	
Skin ulcers	48 (45.3)	108 (59.7)	0.56 (0.35–0.91)	0.018	0.21 (0.08–0.59)
Diagnosis					
Colonization	36 (34.0)	83 (45.6)	1	0.053	
Infection	70 (66.0)	99 (54.4)	1.63 (0.99–2.68)		2.26 (0.96–5.31)
Sample site					
Respiratory	18 (17.0)	26 (14.3)	1	0.053	1
Skin	73 (68.8)	108 (59.3)	0.97 (0.50–1.91)		3.03 (0.85–10.73)
Other[d]	15 (14.2)	48 (26.4)	0.45 (0.20–1.04)		0.27 (0.06–1.14)

Data are presented as *n* (%) unless otherwise indicated.
CI, confidence interval; MRSA, methicillin-resistant *Staphylococcus aureus;* OR, odds ratio; Tet^R, tetracycline resistant; Tet^S, tetracycline susceptible.
[a] Modified Charlson index by age.
[b] At least one antibiotic received during the last 6 months.
[c] Comorbidites include cerebrovascular disease, chronic cardiovascular disease, liver disease, chronic lung disease, chronic renal disease, autoimmune disease, neurologic comorbidity, diabetes and malignancy.
[d] Other includes culture of blood, bile, urine, bone and joint prostheses.

pneumonia, prosthetic joint infection and septic arthritis. These findings were similar to those of other reports [10,11] and suggested that MRSA-Tet^R, and particularly MRSA CC398, increasingly had the same capacity to lead to infection as MRSA-Tet^S, and that these infections had the capacity to develop into severe or high-mortality illnesses.

Nonetheless, there are some significant clinical and epidemiologic differences ($p < 0.001$) between MRSA-Tet^R and MRSA-Tet^S patients. This could be of special relevance for clinicians, as is also the association of MRSA-Tet^R with the following: higher community acquisition, pig farming, fewer comorbidities (lower Charlson index and high Barthel index), limited hospital admissions in the preceding 12 months, less frequent use of antibiotics, almost no contact with nursing homes and less mortality at 6 months (but not at <30 days). A simple resistance phenotype of MRSA strains (Tet^R) could give relevant information for patient management.

We also analysed the antibiotic resistance phenotypes in MRSA of the different groups; we detected greater resistance to erythromycin and especially to clindamycin in MRSA-Tet^R strains. A similar resistance profile was identified in a previous study on pig farm workers [6] and could be associated with the enrichment in clindamycin resistance genes (specially *lnuA, lunB* and *vga*) among MRSA CC398 isolates [5].

The present study's primary limitation is that it only identifies people who demonstrated infection/colonization. With the inclusion of a screening program for the search of carriers in patients on admission to acute care hospital, the prevalence could likely increase.

In summary, the increase in MRSA-Tet^R detection, especially CC398, over the last years suggests that it might become the most prevalent community-associated MRSA clone in our area in the future. Clinical and epidemiologic differences were identified among MRSA-Tet^R and MRSA-Tet^S patients, and severe MRSA CC398 infections were also detected.

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Transparency Declaration

All authors report no conflicts of interest relevant to this article.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2017.03.019.

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