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CODA - CERVA

Antimicrobial resistance

in methicillin-resistant *Staphylococcus aureus*

from cattle in 2015 in Belgium

Report on the occurrence of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* from cattle in 2015 in Belgium.

Summary

The overall MRSA prevalence in cattle in 2015 was 41.0%. This elevated level is caused by the high prevalence in veal calves (78.2%), whereas for bovines for meat production (16.5%) and for dairy cows (10.4%) more moderate levels of MRSA were seen. The higher MRSA prevalence in 2015 than in 2012 might be because of a higher sensitivity of the used isolation method in 2015 and should therefore be interpreted with caution. MRSA ST398, mainly associated with livestock animals, was the predominant sequence type in all categories. A change in *spa*-types could be seen between 2012 and 2015, suggesting a changing profile according to adaptations of the animal host. Among MRSA strains from cattle, in 2015, resistance was detected for all antimicrobials tested, except for linezolid, mupirocin and vancomycin. As expected, all strains were resistant to ceftiofur and penicillin. Resistance to ciprofloxacin in 2015 showed a worrisome increase compared to its level in 2012 (54.9% and 16.0% in 2015 and 2012 respectively). A high prevalence of multi-resistance could be observed in cattle. In addition to the ceftiofur and penicillin resistance, resistance was present to maximum 10 antimicrobials in bovines for meat, and to maximum 13 antibiotics in veal calves and dairy cattle.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognised as an important cause of infections in humans for decades. Strains of MRSA causing infections in humans can be divided into three broad categories, healthcare-associated (HA-), community-associated (CA-) and livestock-associated (LA-) MRSA. LA-MRSA has been detected in pigs, poultry, bovines, horses and dogs and LA-strains have been shown to be distinct from human-derived strains (Fluit, 2012). HA-MRSA and CA-MRSA include strains which predominantly affect humans, yet, there is also an exchange of strains between the reservoirs (Fluit, 2012). LA-MRSA may therefore also be harbored by humans and cause illness in humans. Pigs are often carriers of LA-MRSA, but are only rarely infected (Meemken et al., 2010). In chickens, several disease manifestations have been described (McNamee and Smyth, 2000). Staphylococcal mastitis has been reported in dairy industry (Vanderhaeghen et al., 2010a). In Belgium, in 2014, a 3-fold decrease in the incidence of nosocomial MRSA is seen since 2003 (WIV-ISP, 2015). Also, the proportion of MRSA strains out of the clinical *S. aureus* strains showed a decrease of 14% between 2003 and 2014 (WIV-ISP, 2015). At the European level, a significantly decreasing trend of human-derived MRSA was observed from 2011 to 2014. Yet, MRSA remains a human public health priority, as the percentage of MRSA remains above 25.0% in 7 out of 29 EU countries. However, in Belgium, a decreasing trend of 4% has been observed between 2011 and 2014 (EFSA and ECDC, 2016).

In the framework of the surveillance by Federal Agency for the Safety of the Food Chain (FASFC), a surveillance of MRSA is executed, in order to determine the prevalence and diversity of MRSA strains isolated from production animals. The surveillance consists of a cycle of three years. Pigs were monitored in 2013, poultry in 2014 and bovines in 2015. In this report, prevalence and antimicrobial susceptibility data are presented for MRSA isolated from bovines, more precisely from veal calves, bovines for meat production and dairy cows.

Materials and methods

Sampling

Veal calves, bovines for meat production younger than 7 months and dairy cows with at least one calving were included in the sampling frame.

Veal calves

In total, 147 veal calf herds were visited and sampled. For all sampled farms, 10 nasal swabs from 10 different calves were pooled per farm.

Bovines and dairy cows

In total, 103 herds with bovines for meat production younger than 7 months and 96 herds with dairy cows were visited and sampled. For all sampled farms, 20 nasal swabs from 20 different animals were pooled per farm.

Isolation and identification

Pooled samples were incubated in Mueller-Hinton (MH) broth (Becton Dickinson) supplemented with NaCl (6.5%) at 37°C for 18-24h. One ml of this broth was added to Tryptic Soy Broth (TSB) supplemented with cefoxitin (3.5 mg/l) and aztreonam (75 mg/l) and incubated at 37°C for 18-24h. Ten microliter of this enrichment was plated on Brilliance MRSA 2 (Oxoid) and incubated 18-24h at 37°C. Presence of MRSA was suspected based on colony morphology and confirmed using a triplex real-time PCR method.

Confirmation by real-time PCR

Per sample, one to five suspected colonies were selected from the Brilliance MRSA 2 plate. DNA was extracted as described in SOP/BAC/ANA/18. MRSA confirmation was performed using a triplex real-time PCR method. This PCR allows detecting the Staphylococcal aureus specific gene, *nuc*, the presence of the *mecA* gene responsible for methicillin resistance and the variant *mecC* gene.

Genotyping

Spa typing

All MRSA isolates were *spa*-typed by sequencing the repetitive region of the *spa* gene encoding for the staphylococcal protein A. This method depicts the rapid evolution, since through recombination, the repeats may change fast. The protein A (*spa*) gene was amplified according to the Ridom StaphType standard protocol (www.ridom.de/staphtype) and the amplification was checked on a 2% agarose gel. Sequencing was performed with an ABI capillary instrument using standard protocols and sequences were compared with the international Ridom database.

CC398 PCR

CC398 PCR was performed on all MRSA following protocol described by Stegger *et al.* 2011. This method allows the rapid detection of the *S. aureus* sequence type ST398.

Antimicrobial susceptibility testing

Antimicrobial resistance was determined using the micro broth dilution method (Sensititre, Trek Diagnostics Systems, Magellan Biosciences) following the manufacturer's instructions (SOP/BAC/ANA/11) and using the epidemiological cut-off's (ECOFFs), established by the European Committee on Antimicrobial Susceptibility (EUCAST) or as defined by the EU reference laboratory on antimicrobial resistance (DTU) for *S. aureus*. Samples were first inoculated on a blood agar plate and incubated at 37°C for 24 hours. Three to five colonies from the agar plate were then added in 4 ml of sterile physiological water and adjusted to 0.5 McFarland. Ten microliter of this suspension was inoculated in a tube containing 11ml cation adjusted MuellerHinton broth with TES (Trek Diagnostics). Fifty µl of this inoculum was then inoculated per well using the AIM™ Automated Inoculation Delivery System and incubated at 37°C for 24 hours. Sensititre plates were read with Sensititre Vision System® for semi-automatic registration of the Minimum Inhibitory Concentration (MIC) of the different antimicrobials tested. The MIC was defined as the lowest concentration by which no visible growth could be detected.

Table 1 : Panel of antimicrobial substances included in antimicrobial susceptibility testing, concentration ranges tested and EUCAST epidemiological cut-off's (ECOFFs) for methicillin resistant *Staphylococcus aureus*

Antimicrobial (Abbreviation)	Concentration range, mg/l	EUCAST ECOFF
Chloramphenicol (CHL)	4-64	> 16
Ciprofloxacin (CIP)	0.25-8	> 1
Clindamycine (CLI)	0.12-4	> 0.25
Erythromycine (ERY)	0.25-8	> 1
Cefoxitin (FOX)	0.5-16	> 4
Fusidic acid (FUS)	0.5-4	> 0.5
Gentamicin (GEN)	1-16	> 2
Kanamycine (KAN)	4-64	> 8
Linezolid (LZD)	1-8	> 4
Mupirocin (MUP)	0.5-256	> 1
Penicillin (PEN)	0.12-2	> 0.12
Rifampicin (RIF)	0.016-0.5	> 0.03
Sulfamethoxazole (SMX)	64-512	> 128
Streptomycin (STR)	4-32	> 16
Quinupristin/dalfopristin (SYN)	0.5-4	> 1
Tetracycline (TET)	0.5-16	> 1
Tiamulin (TIA)	0.5-4	> 2
Trimethoprim (TMP)	2-32	> 2
Vancomycin (VAN)	1-16	> 2

EUCAST: European Committee on Antimicrobial Susceptibility Testing

Data analysis and description

Data from the Excel file generated by the software of the semi-automated susceptibility equipment (sensivision, Trek Diagnostics) were incorporated in the LIMS system at CODA-CERVA together with the metadata associated with the sampling. These files were validated for consistency.

Isolates with a MIC value higher than the ECOFF value were considered not to belong to the wild type population and percentages of isolates with a reduced susceptibility, i.e. non-wild type, were calculated. Throughout the report, isolates with a reduced susceptibility will be referred to as 'resistant isolates', whereas when the clinical interpretative criterion was used, the term 'clinical resistance' will be used.

The number of antimicrobials to which a strain was resistant was counted and cumulative percentages or percentiles were calculated. Graphical representations were prepared in Excel.

Throughout the report, terms used to describe the levels or occurrence of antimicrobial resistance are those proposed by EFSA. Rare: <0.1%', 'very low: >0.1% to 1.0%', 'low: >1% to 10.0%', 'moderate: >10.0% to 20.0%', 'high: >20.0% to 50.0%', 'very high: >50.0% to 70.0%', 'extremely high: >70.0%'. Although these terms are applied to all antimicrobials, the significance of a given level of resistance will depend on the particular antimicrobial and its importance in human and veterinary medicine.

A multi-resistant isolate is one defined as resistant to at least three different antimicrobial substances, included in the analysis (Table 1). It should be noted that all confirmed MRSA strains should show resistance to minimum 2 antibiotics, cefoxitin and penicillin.

Statistical analysis

The number of resistant strains was counted and resistance percentages were calculated. Exact confidence intervals for the binomial distribution were calculated using a VBA script in Excel. A 95% symmetrical two-sided confidence interval was used with $p=0.025$. The lower and upper bound of confidence interval for the population proportion was calculated. Based on the Pearson's chi-square test, and where appropriate the Fischer exact test, significance of the differences were calculated.

Results

Prevalence of Methicillin Resistant *Staphylococcus aureus* and the sequence type ST398

The presence of MRSA was confirmed for 142 strains out of the 346 analyzed samples (41.0%), based on real-time PCR, for all animal categories. For veal calves, bovines for meat and dairy cows, 78.2%, 16.5% and 10.4% samples were MRSA positive respectively. Based on confidence intervals, no significant difference between bovines for meat and dairy cows was present (Table 2).

Among 142 MRSA strains recovered, 108 (76.1%) were positive for the cc398 PCR and considered as MRSA sequence type ST398. The sequence type ST398 was present in 75.7% of the MRSA strains of veal calves, in 76.5% of the MRSA strains of bovines for meat and in 80.0% of MRSA strains of dairy cows. The low number of samples does not allow detecting significant differences of the presence of the sequence type ST 398 between the different animal categories (Table 2).

Table 2 : Prevalence of Methicillin Resistant *Staphylococcus aureus* and sequence type ST398 in veal calves, bovines for meat and dairy cows

Herd type	Number of visited herds	MRSA positive (%) 95% Confidence Interval	Number of MRSA strains positive for sequence type ST398 (%) 95% Confidence Interval
Veal calves	147	115 (78.2%) 70.7-85.0%	87 (75.7%) 66.8-83.0%
Bovines for meat	103	17 (16.5%) 9.9-25.0%	13 (76.5%) 50.1-93.0%
Dairy cows	96	10 (10.4%) 5.1-18.0%	8 (80.0%) 44.4-97.0%
Total	346	142 (41.0%) 35.8-46.0%	108 (76.1%) 68.2-83.0%

Characterization of Methicillin Resistant *Staphylococcus aureus*

Nine different *spa*-types were identified. Seventy-seven (54.2%) were *spa*-type t011, and less frequently occurred the *spa*-types t034, t037, t044, t1451, t1580, t1985, t2287, t2383, t3423 and non-typable (NT). MRSA ST398 was associated to all identified *spa*-types, except t1451 and t2383. All t011 *spa*-types were associated with MRSA ST398. Thirty-four MRSA strains were different from ST398. Among these MRSA strains the following *spa*-types were found: t034, t037, t044, t1451, t1580, t2383, t3423.

Table 3 : Total number of Methicillin Resistant *Staphylococcus aureus* in veal calves, bovines for meat and dairy cows corresponding to the different genotypes

Herd type	<i>spa</i> -types											Total
	t011	t034	t037	t044	t1451	t1580	t1985	t2287	t2383	t3423	NT	
veal calves	63	15	8	3	3	7	8	2	0	5	1	115
bovines for meat	10	2	0	0	1	2	0	1	0	1	0	17
dairy cows	4	1	0	0	0	1	2	0	1	0	1	10
Total	77	18	8	3	4	10	10	3	1	6	2	142

NT: non-typable

Antimicrobial resistance of Methicillin Resistant *Staphylococcus aureus*

Antimicrobial resistance occurrence for the confirmed MRSA strains for the different categories is presented in the figures below.

As expected, all MRSA strains were resistant to ceftiofur and penicillin, except for one strain in dairy cows which showed susceptibility to ceftiofur. This should be regarded as a methodological deviation since the presence of the *mecA* gene was demonstrated.

Veal calves

Antimicrobial resistance was at extremely high levels for clindamycin, tetracycline, erythromycin, and trimethoprim; at very high levels for kanamycin, gentamicin and ciprofloxacin; at high levels for streptomycin and at moderate levels for quinupristin/dalfopristin. For sulfamethoxazole, chloramphenicol, tiamulin, fusidic acid and rifampicin, antimicrobial resistance levels remained low, whereas for linezolid, mupirocin and vancomycin antimicrobial resistance remained undetected (Figure 1).

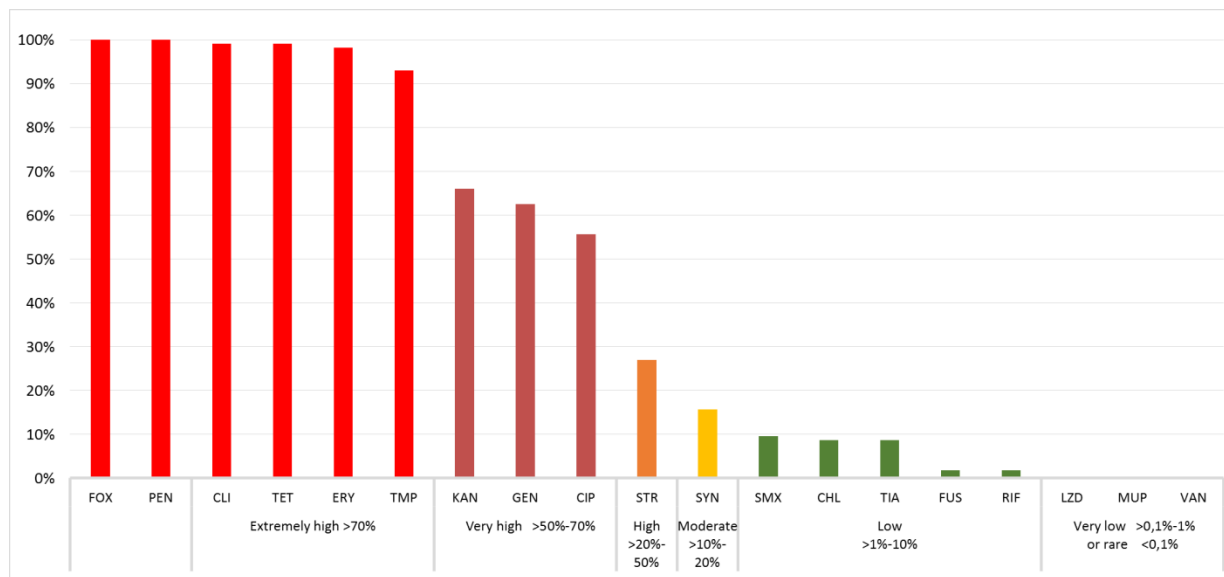


Figure 1 : Antimicrobial resistance prevalence for methicillin resistant *Staphylococcus aureus* (n= 115), isolated from veal calves at the farm, based on epidemiological cut-off's, according to the European Committee on Antimicrobial Susceptibility (EUCAST) for ceftiofur (FOX), penicillin (PEN), clindamycin (CLI), tetracycline (TET), erythromycin (ERY), trimethoprim (TMP), kanamycin (KAN), gentamicin (GEN), ciprofloxacin (CIP), streptomycin (STR), quinupristin/dalfopristin (SYN), sulfamethoxazole (SMX), chloramphenicol (CHL), tiamulin (TIA), fusidic acid (FUS), rifampicin (RIF), linezolid (LIN), mupirocin (MUP), vancomycin (VAN).

Multiple antimicrobial resistance patterns of Methicillin Resistant *Staphylococcus aureus* in veal calves

All confirmed MRSA strains showed resistance to minimum 2 antibiotics, ceftiofur and penicillin, and these resistances were not included in the multi-resistance patterns.

In veal calves, MRSA showed resistance to a least 3 other antimicrobials (next to ceftiofur and penicillin) and were mainly resistant to 5 (22.6%), 6 (24.3%), 7 (19.1%) or 8 other antimicrobial agents (21.7%). One strain (ST398, t011) showed resistance to 13 antibiotics (0.9%) (Figures 4 and 5).

Bovines

Antimicrobial resistance was at extremely high levels for tetracycline, trimethoprim, gentamicin and kanamycin; at very high levels for clindamycin and ciprofloxacin, and at high levels for erythromycin, sulfamethoxazole, streptomycin, quinupristin/dalfopristin and tiamulin. Moderate levels were seen for fusidic acid, whereas for chloramphenicol, linezolid, mupirocin, rifampicin and vancomycin antimicrobial resistance levels remained undetected (Figure 2).

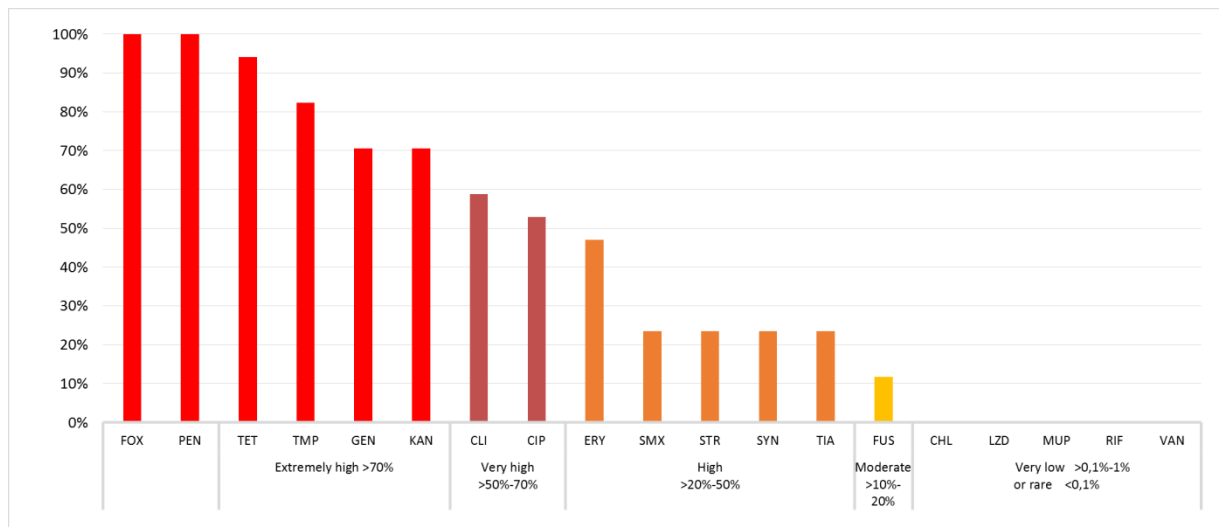


Figure 2 : Antimicrobial resistance prevalence for methicillin resistant *Staphylococcus aureus* (n= 17), isolated from bovines for meat younger than 7 months at the farm, based on epidemiological cut-off's, according to the European Committee on Antimicrobial Susceptibility (EUCAST) for ceftiofur (FOX), penicillin (PEN), clindamycin (CLI), tetracycline (TET), erythromycin (ERY), trimethoprim (TMP), kanamycin (KAN), gentamicin (GEN), ciprofloxacin (CIP), streptomycin (STR), quinupristin/dalfopristin (SYN), sulfamethoxazole (SMX), chloramphenicol (CHL), tiamulin (TIA), fusidic acid (FUS), rifampicin (RIF), linezolid (LIN), mupirocin (MUP), vancomycin (VAN).

Multiple antimicrobial resistance patterns of Methicillin Resistant *Staphylococcus aureus* in bovines for meat production

All confirmed MRSA strains showed resistance to minimum 2 antibiotics, ceftiofur and penicillin, and these resistances were not included in the multi-resistance patterns.

In bovines for meat production, MRSA strains showed resistance to a least 3 other antimicrobials (next to ceftiofur and penicillin) and were mainly resistant to 7 antimicrobial substances (4 out of 17 strains, 23.5%). One strain (ST398, t011) showed resistance to 10 antibiotics, remaining susceptibility only to chloramphenicol, erythromycin, linezolid, mupirocin, rifampicin, trimethoprim and vancomycin (Figures 4 and 5).

Dairy cows

Antimicrobial resistance was at extremely high levels for tetracycline and trimethoprim; at very high levels for ciprofloxacin, gentamicin, kanamycin, clindamycin, erythromycin and streptomycin, and at moderate levels for fusidic acid, quinupristin/dalfopristin, tiamulin, rifampicin and sulfamethoxazole. For chloramphenicol, linezolid, mupirocin and vancomycin antimicrobial resistance levels remained undetected (Figure 3).

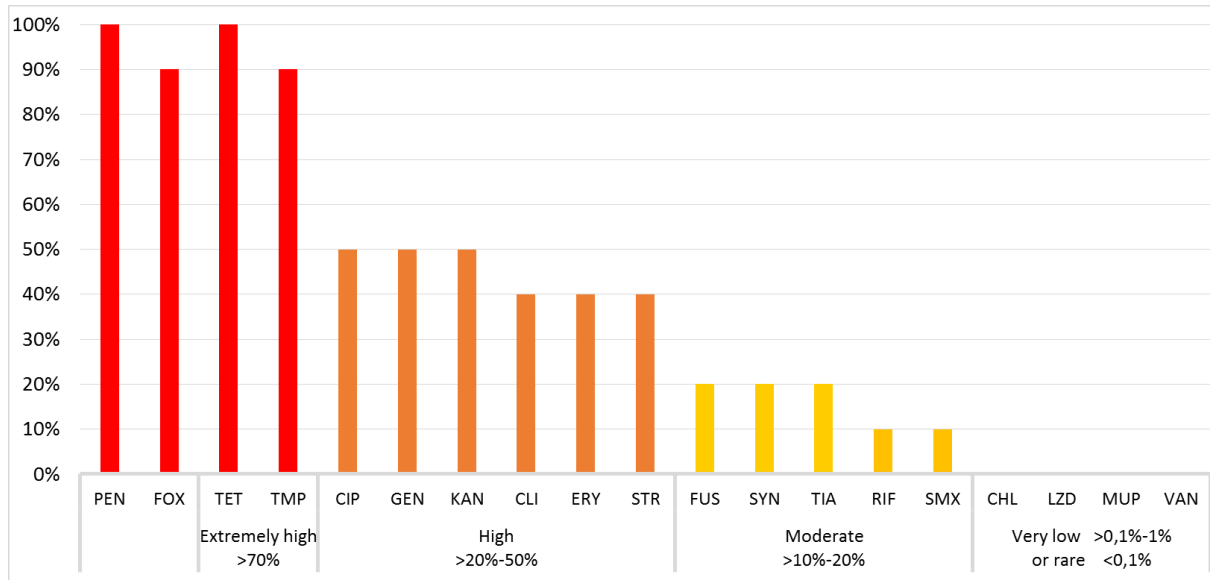


Figure 3 : Antimicrobial resistance prevalence for methicillin resistant *Staphylococcus aureus* (n= 10), isolated from dairy cows at the farm, based on epidemiological cut-off's, according to the European Committee on Antimicrobial Susceptibility (EUCAST) for cefoxitin (FOX), penicillin (PEN), clindamycin (CLI), tetracycline (TET), erythromycin (ERY), trimethoprim (TMP), kanamycin (KAN), gentamicin (GEN), ciprofloxacin (CIP), streptomycin (STR), quinupristin/dalfopristin (SYN), sulfamethoxazole (SMX), chloramphenicol (CHL), tiamulin (TIA), fusidic acid (FUS), rifampicin (RIF), linezolid (LIN), mupirocin (MUP), vancomycin (VAN).

Multiple antimicrobial resistance patterns of Methicillin Resistant *Staphylococcus aureus* in dairy cows

All confirmed MRSA strains showed resistance to minimum 2 antibiotics, cefoxitin and penicillin, and these resistances were not included in the multi-resistance patterns.

In dairy cows, MRSA strains showed resistance to a least 1 other antimicrobial (next to cefoxitin and penicillin) and were mainly resistant to 5 antimicrobial substances (3 out of 10 strains, 30.0%). One strain showed resistance to 13 other antibiotics (different from ST398, t2383) (Figures 4 and 5).

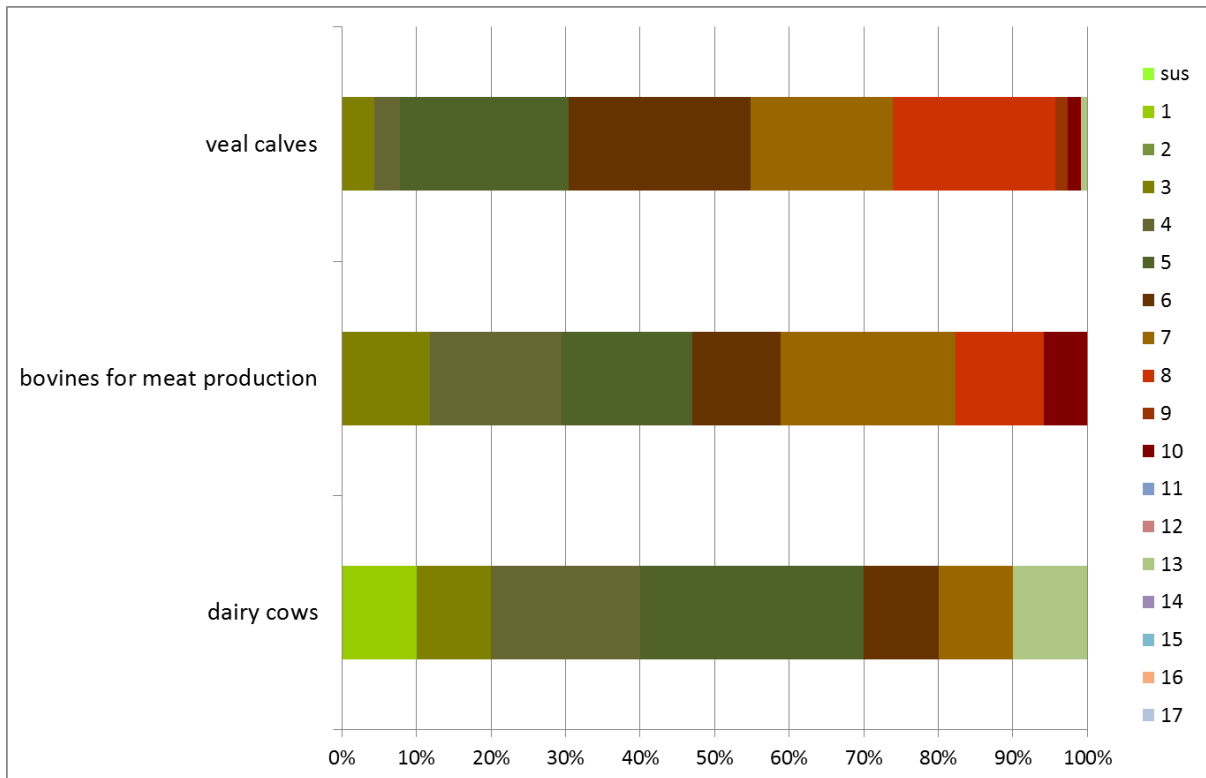


Figure 4 : Percentages of Methicillin Resistant *Staphylococcus aureus* from veal calves (n= 115), bovines for meat production (n= 17) and dairy cows (n= 10) showing full susceptibility or resistance to at least 1 antimicrobial. Resistance to ceftaxime and ceftazidime are not included.

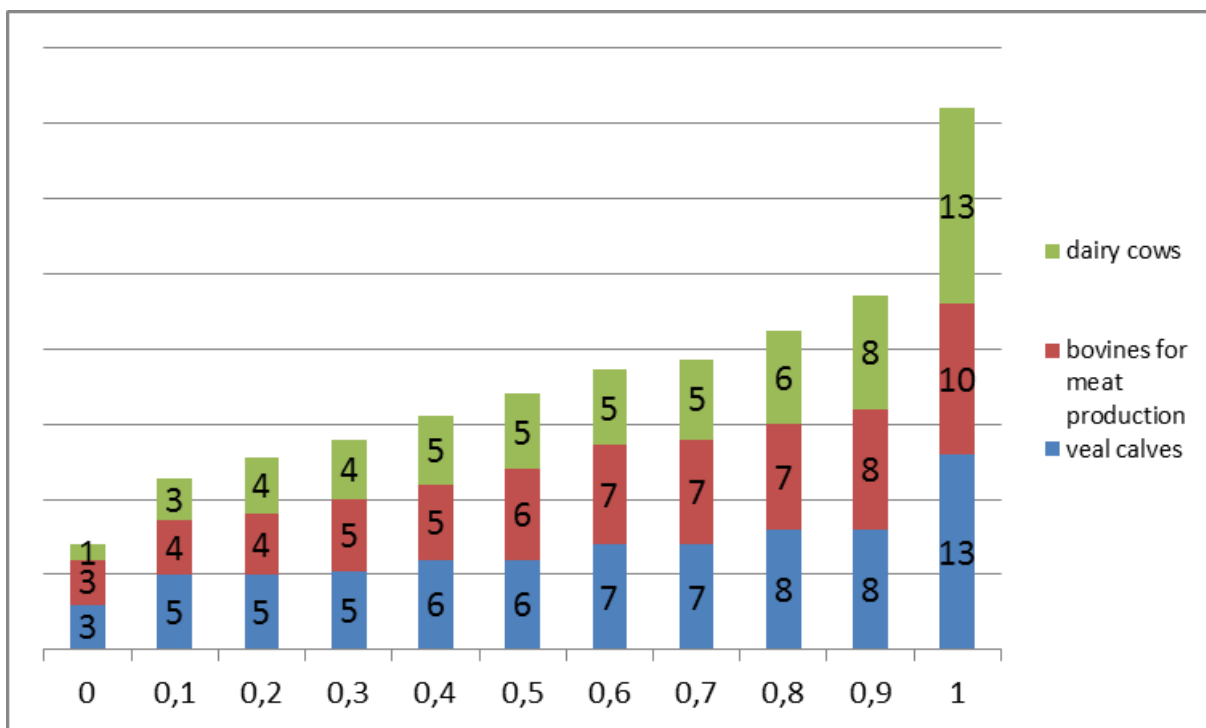


Figure 5 : Percentiles of Methicillin Resistant *Staphylococcus aureus* from veal calves (n= 115), bovines for meat production (n= 17) and dairy cows (n= 10) showing full susceptibility or resistance to at least 1 antimicrobial. Resistance to nalidixic acid and resistance to ciprofloxacin, as well as the resistance to ceftaxime and ceftazidime are not included.

Discussion

The overall MRSA prevalence in cattle in 2015 was 41.0%. This elevated level is caused by the high prevalence in veal calves (78.2%), whereas for bovines for meat production (16.5%) and for dairy cows (10.4%) more moderate levels of MRSA were seen. MRSA ST398, mainly associated with livestock animals, was the predominant sequence type in all categories. No further MLST subtyping was conducted. Therefore, sequence types classified among hospital-acquired (HA-) or community-acquired (CA-) MRSA, could not be identified. In view of reports on possible spreads of HA-MRSA to livestock, sequence typing is of critical relevance (Smith, 2015). As in MRSA collected from cattle in 2012, the main *spa*-type was t011 and all were associated with MRSA ST398. Other less prevalent *spa*-types were also recovered. Five additional *spa*-types were seen compared to 2012 (t034, t044, t1580, t2287 and t2383), whereas four *spa*-types seen in 2012 were not observed anymore in 2015 (t121, t388, t1456, t6228). It was mainly in veal calves that a larger diversity of *spa*-types was observed (CODA-CERVA, 2012). A change in *spa*-types reflects adaptations of MRSA to its host and might indicate that host adaptations are underway (Kahl et al., 2005). The new *spa*-types identified in cattle in 2015 (t034, t044 and t1580), were not solely associated to ST398. MRSA *spa*-type t044 has been shown to be associated to ST80, which belongs to the CA-MRSA (<http://spa.ridom.de/>). This confirms the spread to livestock of MRSA originating from humans and an adaptation of the strains to an animal host.

MRSA prevalence in bovines for meat (16.5%) was slightly higher than in dairy cows (10.4%). In Belgium, a higher MRSA prevalence in bovines for meat has been reported before (Vandendriessche et al., 2013). MRSA ST398 has been shown to be implicated in mastitis in dairy cows and therefore measures should be taken to eliminate these MRSA strains (Vanderhaeghen et al., 2010a).

MRSA prevalence in all cattle categories is generally higher than in poultry, for which the level of occurrence was 2.45% in 2014, using identical isolation methods (total number of samples was 326) (CODA-CERVA, 2014). The high level of MRSA in veal calves surpasses the MRSA presence in fattening pigs in 2013 (65.5% out of 328 samples), but less sensitive methods were then used (CODA-CERVA, 2013). Nevertheless, a higher prevalence in veal calves than in fattening pigs has been reported before when using identical methods (Vandendriessche et al., 2013). Veal calf herds need to purchase animals from a great variety of herds or through markets or traders. This increases the risk that at least 1 of the calves originates from a MRSA-positive dairy farm. Moreover, a different level of antimicrobial use in veal calves could contribute to the differences. Currently, antimicrobial use is not being monitored in cattle. Yet, the diverse origin of veal calves raised on veal farms and the disease conditions associated with crowding is known to increase antimicrobial use in veal calves (Pardon et al., 2012).

MRSA prevalence data in cattle for 2015 are hard to compare with the 2012 data because of a higher sensitivity of the used isolation method in 2015 (CODA-CERVA, 2012). Therefore, the higher MRSA prevalence in 2015 than in 2012 for veal calves (78.2% and 47.1% respectively) and for bovines for meat (16.5% in 2015 and 10.2% in 2012) should be interpreted with caution. For dairy cows, only a very slight increase can be observed between 2012 and 2015 (9.9% in 2012 and 10.4% in 2015). In general, for all cattle categories, the similar proportional increase in the MRSA prevalence between 2012 and 2015, might affirm the observed differences as a result of different isolation methods. Also, unequal sample sizes between 2012 and 2015 should be taken into account when comparing the data (for veal calves: 104 and 147 samples in 2012 and 2015 respectively; for bovines for meat production: 187 and 103 in 2012 and 2015 respectively; for dairy cattle: 141 and 96 in 2012 and 2015 respectively).

Among MRSA isolates from cattle, in 2015, resistance was detected for all antimicrobials tested, except for linezolid, mupirocin and vancomycin. Antimicrobial resistance to tetracycline was common, with only two isolates susceptible to this antimicrobial. Tetracycline resistance is typically associated with LA-MRSA, belonging to sequence type ST398, and is due to the presence of the *tet(M)* gene on a chromosomally located transposon, often in combination with the plasmid-encoded *tet(K)* gene (Crombé et al., 2012; Crombé et al., 2013). Susceptibility to tetracycline in MRSA has previously been found, despite the presence of resistance genes (Verhegge et al., 2016). Antimicrobial resistance genes can be suppressed or expressed at a lower level, resulting in the absence of phenotypic (Verhegge et al., 2016). Likewise, resistance to trimethoprim is widespread by the presence of the *drfK* gene and trimethoprim susceptible strains are only very rarely found (Kadlec et al., 2012). In this monitoring study, 8 MRSA strains from veal calves, 3 MRSA strains from bovines for meat and 1 MRSA strain from dairy cows were found susceptible to trimethoprim.

Resistance to clindamycin and erythromycin remained high, compared to 2012, whereas for kanamycin and gentamicin a decrease of 10% could be seen. For ciprofloxacin, a critically important antimicrobial for human and veterinary medicine, resistance was approximately 50% in all cattle categories. Moreover, resistance to ciprofloxacin in 2015 showed a worrisome increase compared to its level in 2012 (54.9% and 16.0% in 2015 and 2012 respectively). Ciprofloxacin resistance was associated with resistance to at least 3 other antibiotics, but also resistance to 5 or 8 antimicrobials was seen (β -lactam resistance of MRSA not included). One strain from veal calves (t011) was even resistant to 13 other antibiotics.

For other antibiotics, i.e. fusidic acid, mupirocin, rifampicin, sulfamethoxazole, streptomycin, quinupristin/dalfopristin and tiamulin a decrease was observed compared to 2012 (CODA-CERVA, 2012). In 2012, a first estimation on antimicrobial use in veal calves, was performed, but no new data have appeared since then. A raise or decrease in antimicrobial resistance can therefore not be associated with the use of antimicrobial agents.

In this monitoring study, resistance to chloramphenicol was present in veal calves and not in bovines for meat or in dairy cows. Chloramphenicol resistance also has not been detected in MRSA associated with mastitis in dairy cows in Belgium (Vanderhaeghen et al., 2010a). Resistance to chloramphenicol and other phenicols (e.g. florfenicol) in MRSA is exerted by the presence of the *fexA* gene, coding for an efflux pump, and is expressed inducible by the use of florfenicol and chloramphenicol (Kadlec et al., 2012). Also, the multi-resistance gene *cfr*, mostly located on plasmids, confers resistance to phenicols, as well as to many other antimicrobial agents in MRSA (Kadlec et al., 2012). The presence of the *fexA* or *cfr* gene has been shown in phenicol resistant LA-MRSA ST398 strains isolated from pigs and cattle (Kadlec et al., 2012). The high florfenicol use in veal calves might thus select for these genes and confers therefore cross-resistance with chloramphenicol (Pardon et al., 2012), whereas florfenicol is not registered, for cows producing milk for human consumption.

Resistance to linezolid, mupirocin and vancomycin was not detected in MRSA isolated of any of the cattle categories. Linezolid and vancomycin are both antimicrobials of last resort for treating *S. aureus* infections in humans and resistance to them is currently extremely rare. Mupirocin is not licensed in animals and is used for topical treatment and decolonization of MRSA in the nose of human patients (Coates et al., 2009). Cross-resistance with other antimicrobials does not occur, due to mupirocin's novel mechanism of action (Cookson, 1998), but the *MupA* gene, conferring mupirocin resistance, may co-transfer with other antibacterial resistance genes, i.e. tetracycline and trimethoprim (Dowling, 2013). MRSA isolated from cattle in 2012 still showed 10% resistance to

mupirocin, whereas in this study resistance was not any longer detected in any of the three cattle categories.

In 2012, in cattle, an overall resistance to rifampicin of 13.6% was observed. Although in 2015, the overall rifampicin resistance in cattle decreased to 2.1%, a 10% resistance was still present in MRSA from dairy cows and 1.7% resistance in veal calves. Only MRSA from bovines for meat production showed full susceptibility to this antimicrobial. A 10% resistance in dairy cows can still be considered as low, yet, it is a substantial increase compared to MRSA associated with mastitis in cows in Belgium. In pigs and poultry, rifampicin resistance is generally observed (CODA-CERVA, 2013-2014). All MRSA strains were resistant to at least 3 antimicrobials, in addition to the ceftiofur and penicillin resistance typically related to MRSA. A maximum of resistance to 13 antimicrobials was seen in one MRSA strain isolated from both veal calves and dairy cows. In bovines for meat, resistance to maximum 10 antimicrobials was seen. Therefore, a high prevalence of multi-resistance could be seen in cattle. Antimicrobial resistance genes in LA-MRSA are often located on plasmids, resulting in multi-resistant LA-MRSA strains (Kadlec et al., 2012). The co-localization of these resistance genes with other resistance genes enables their co-selection and persistence. LA-MRSA can therefore act as a donor and a recipient of antimicrobial resistance genes within the Gram-positive gene pool.

Supplementary data

Table 4: Minimum Inhibitory Concentrations for methicillin-resistant *Staphylococcus aureus* strains (n= 115), isolated from veal calves, for chloramphenicol (CHL), ciprofloxacin (CIP), clindamycine (CLI), erythromycin (ERY), ceftiofur (FOX), fusidic acid (FUS), gentamicin (GEN), kanamycine (KAN), linezolid (LZD), mupirocin (MUP), penicillin (PEN), rifampicin (RIF), sulfamethoxazole (SMX), streptomycin (STR), quinupristin/dalfopristin (SYN), tetracycline (TET), tiamulin (TIA), trimethoprim (TMP) and vancomycin (VAN). Epidemiological cut-off's (ECOFFs) are indicated as straight (|) lines.

	<=0.016	<=0.03	<=0.06	<=0.12	<=0.25	<=0.5	<=1	<=2	<=4	<=8	16	32	64	128	256	512	1024	2048
CHL	-	-	-	-	-	-	-	-	2	82	21	1	7	2	-	-	-	-
CIP	-	-	-	-	20	24	8	4	0	28	31	-	-	-	-	-	-	-
CLI	-	-	-	1	0	0	0	0	0	114	-	-	-	-	-	-	-	-
ERY	-	-	-	-	2	0	0	0	0	0	113	-	-	-	-	-	-	-
FOX	-	-	-	-	-	0	0	0	0	20	45	50	-	-	-	-	-	-
FUS	-	-	-	-	-	113	0	0	0	2	-	-	-	-	-	-	-	-
GEN	-	-	-	-	-	-	42	0	3	14	28	28	-	-	-	-	-	-
KAN	-	-	-	-	-	-	-	-	36	3	4	3	13	56	-	-	-	-
LZD	-	-	-	-	-	-	25	90	0	0	0	-	-	-	-	-	-	-
MUP	-	-	-	-	-	113	2	0	0	0	0	0	0	0	0	0	-	-
PEN	-	-	-	0	0	0	0	1	114	-	-	-	-	-	-	-	-	-
RIF	113	0	0	0	0	0	2	-	-	-	-	-	-	-	-	-	-	-
SMX	-	-	-	-	-	-	-	-	-	-	-	-	102	2	0	0	11	-
STR	-	-	-	-	-	-	-	-	59	20	5	6	25	-	-	-	-	-
SYN	-	-	-	-	-	23	74	11	3	4	-	-	-	-	-	-	-	-
TET	-	-	-	-	-	1	0	0	0	0	0	114	-	-	-	-	-	-
TIA	-	-	-	-	-	104	1	0	1	9	-	-	-	-	-	-	-	-
TMP	-	-	-	-	-	-	-	8	0	0	0	0	107	-	-	-	-	-
VAN	-	-	-	-	-	-	114	1	0	0	0	0	0	-	-	-	-	-

Table 5: Minimum Inhibitory Concentrations for methicillin-resistant *Staphylococcus aureus* strains (n= 17), isolated from bovines for meat, for chloramphenicol (CHL), ciprofloxacin (CIP), clindamycine (CLI), erythromycin (ERY), ceftiofur (FOX), fusidic acid (FUS), gentamicin (GEN), kanamycine (KAN), linezolid (LZD), mupirocin (MUP), penicillin (PEN), rifampicin (RIF), sulfamethoxazole (SMX), streptomycin (STR), quinupristin/dalfopristin (SYN), tetracycline (TET), tiamulin (TIA), trimethoprim (TMP) and vancomycin (VAN). Epidemiological cut-off's (ECOFFs) are indicated as straight (|) lines.

	<=0.016	<=0.03	<=0.06	<=0.12	<=0.25	<=0.5	<=1	<=2	<=4	<=8	16	32	64	128	256	512	1024	2048
CHL	-	-	-	-	-	-	-	-	3	14	0	0	0	0	-	-	-	-
CIP	-	-	-	-	5	0	2	0	1	2	7	-	-	-	-	-	-	-
CLI	-	-	-	5	2	0	0	0	0	10	-	-	-	-	-	-	-	-
ERY	-	-	-	-	7	2	0	0	0	0	8	-	-	-	-	-	-	-
FOX	-	-	-	-	-	0	0	0	0	2	13	2	-	-	-	-	-	-
FUS	-	-	-	-	-	15	0	1	0	1	-	-	-	-	-	-	-	-
GEN	-	-	-	-	-	-	5	0	0	2	4	6	-	-	-	-	-	-
KAN	-	-	-	-	-	-	-	-	4	1	0	0	1	11	-	-	-	-
LZD	-	-	-	-	-	-	4	13	0	0	0	-	-	-	-	-	-	-
MUP	-	-	-	-	-	15	2	0	0	0	0	0	0	0	0	0	-	-
PEN	-	-	-	0	0	1	0	0	16	-	-	-	-	-	-	-	-	-
RIF	17	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-
SMX	-	-	-	-	-	-	-	-	-	-	-	-	13	0	0	1	3	-
STR	-	-	-	-	-	-	-	-	9	4	0	1	3	-	-	-	-	-
SYN	-	-	-	-	-	10	3	1	3	0	-	-	-	-	-	-	-	-
TET	-	-	-	-	-	0	1	0	0	0	0	16	-	-	-	-	-	-
TIA	-	-	-	-	-	13	0	0	0	4	-	-	-	-	-	-	-	-
TMP	-	-	-	-	-	-	-	3	0	0	0	0	14	-	-	-	-	-
VAN	-	-	-	-	-	-	17	0	0	0	0	0	-	-	-	-	-	-

Table 6: Minimum Inhibitory Concentrations for methicillin-resistant *Staphylococcus aureus* strains (n= 10), isolated from dairy cows, for chloramphenicol (CHL), ciprofloxacin (CIP), clindamycine (CLI), erythromycin (ERY), ceftiofur (FOX), fusidic acid (FUS), gentamicin (GEN), kanamycine (KAN), linezolid (LZD), mupirocin (MUP), penicillin (PEN), rifampicin (RIF), sulfamethoxazole (SMX), streptomycin (STR), quinupristin/dalfopristin (SYN), tetracycline (TET), tiamulin (TIA), trimethoprim (TMP) and vancomycin (VAN). Epidemiological cut-off's (ECOFFs) are indicated as straight (|) lines.

	<=0.016	<=0.03	<=0.06	<=0.12	<=0.25	<=0.5	<=1	<=2	<=4	<=8	16	32	64	128	256	512	1024	2048	
CHL	-	-	-	-	-	-	-	-	2	7	1		0	0	0	-	-	-	-
CIP	-	-	-	-	1	2	2		0	0	3	2	-	-	-	-	-	-	-
CLI	-	-	-	4	2		0	0	1	0	3	-	-	-	-	-	-	-	-
ERY	-	-	-	-	5	1	0		1	0	0	3	-	-	-	-	-	-	-
FOX	-	-	-	-	-	0	0	0	1		2	5	2	-	-	-	-	-	-
FUS	-	-	-	-	-	8		0	1	1	0	-	-	-	-	-	-	-	-
GEN	-	-	-	-	-	-	5	0		1	1	2	1	-	-	-	-	-	-
KAN	-	-	-	-	-	-	-	-	3	2		0	0	1	4	-	-	-	-
LZD	-	-	-	-	-	-	3	7	0		0	0	-	-	-	-	-	-	-
MUP	-	-	-	-	-	9	1		0	0	0	0	0	0	0	0	0	-	-
PEN	-	-	-	0		0	0	0	1	9	-	-	-	-	-	-	-	-	-
RIF	9	1		0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-
SMX	-	-	-	-	-	-	-	-	-	-	-	-	9	0		0	0	1	-
STR	-	-	-	-	-	-	-	-	6	0	0		2	2	-	-	-	-	-
SYN	-	-	-	-	-	5	3		1	1	0	-	-	-	-	-	-	-	-
TET	-	-	-	-	-	0	0		0	0	0	0	10	-	-	-	-	-	-
TIA	-	-	-	-	-	8	0	0		0	2	-	-	-	-	-	-	-	-
TMP	-	-	-	-	-	-	-	1		0	0	0	0	9	-	-	-	-	-
VAN	-	-	-	-	-	-	10	0		0	0	0	0	-	-	-	-	-	-

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