MRSA surveillance 2014: Poultry

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1. Introduction

In the framework of the FASFC surveillance, a surveillance of MRSA in poultry has been executed in order to determine the prevalence and diversity of MRSA in poultry for the year 2014.

2. Materials and methods

2.1. Isolation

For all sampled farms, maximum 20 nasal swabs were pooled per farm and 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 (3.5mg/l) and aztreonam (75mg/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.

2.2. 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.

2.3. Genotyping

2.3.1. *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 CEQ 8000 using standard protocols and sequences were compared with the international Ridom database.

2.3.1. 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.

2.4. Determination of antimicrobial resistance in MRSA strains by micro-dilution (Sensititre ®)

Antimicrobial resistance was determined using the micro broth dilution method (Sensititre, Trek Diagnisis Systems, Magellan Biosciences) following the manufacturer's instructions (SOP/BAC/ANA/11) and using the EUCAST ECOFF breakpoints 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.

2.5. 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 visual basic application 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 Pearsons chi-square test, and where appropriate the Fischer exact test, significance of the differences were calculated.

3. Results and discussion

Only 8 out of 326 samples (2.45%) were confirmed positive for MRSA, all carrying the *mecA* gene. Compared to previously reported prevalence in Belgium for example at farms harboring bovines or pigs, the prevalence of MRSA is low. Pooling of samples is interesting, though might have a lowered sensitivity when at farm level, prevalence is low. Results of antimicrobial resistance are shown in Table 1. As expected, all strains were resistant to cefoxitin and penicillin. 87.5% of the strains were resistant to tetracycline and erythromycin. A high prevalence of resistance, 62.5%, was also observed for chloramphenicol, kanamycin, rifampicin, sulfamethoxazole and streptomycin. 50% of the strains tested were resistant to clindamycin. Lower resistance levels were detected for trimethoprim (37.5%), ciprofloxacin (25%), fusidic acid (25%), tiamulin (25%), gentamycin (12.5%), mupirocin (12.5%) and quinupristin/dalfopristin (12.5%). No resistances were observed towards linezolid and vancomycin. In this study one strain was found to be susceptible to tetracycline.

Multi-resistance was calculated and results are shown in figure 1 and 2. It should be noted that all strains are resistant to minimum 2 antibiotics, cefoxitin and penicillin as expected. 50% of the strains are resistant to 9 different antibiotics. One strain had resistance to 15 different antibiotics, remaining susceptible only to trimethoprim, ciprofloxacin, linezolid and vancomycin of which the three last antibiotics are last resort antibiotics in the treatment of MRSA infections in humans. In general it can be stated that the level of multi-resistance is extremely high.

Among the eight MRSA strains isolates from poultry in 2014 only three strains were positives for the cc398 PCR and considered as MRSA ST398. Only 2 out of 8 isolates were typical LA-MRSA ST398, *spa*-type t011. The 5 remaining strains were spa-type t037. Four out of five strains being t037 had the same antibiotic resistance profile. MRSA ST239 t037 was recovered for the first time in Belgium among MRSA in poultry during the previous Belgian survey in poultry in 2011. This interesting finding confirms the possible spread of HA-MRSA to livestock hypothesized in 2011. This situation should also be followed up closely since it may be an indication of a new animal adapted MRSA strain originating from humans.

The *spa* gene is a surface protein that is under the selective pressure of host immunity. Differences in this immunity might be the indication that MRSA CC398 is changing its profile according to the host and that some host adaptations are underway.

4. Conclusion

This surveillance confirms the presence of MRSA in poultry (Nemati *et al.* 2009, Persoons *et al.* 2009) however the prevalence in chickens is still low. Concerning antimicrobial resistance, all strains were resistant to at least 6 antimicrobials and to maximum 15 out of 19 antimicrobials tested, indicating a high prevalence of multi-resistance. As expected, all strains were resistant to penicillin and to cefoxitin. None were resistant to linezolid and vancomycin. Four out of five strains being t037 had the same antibiotic resistance profile.

Table 1. Antibiotic resistance in MRSA from poultry

N°	CC398 PCR	*Spa*-type	CHL	CIP	CLI	ERY	FOX	FUS	GEN	KAN	LZD	MUP	PEN	RIF	SMX	STR	SYN	TET	TIA	TMP	VAN
44	-	t037	>64	<=0.25	<=0.12	>8	>16	<=0.5	<=1	>64	2	<=0.5	>2	>0.5	>512	>32	<=0.5	>16	1	<=2	<=1
51	-	t037	64	<=0.25	<=0.12	>8	>16	<=0.5	<=1	>64	<=1	<=0.5	>2	>0.5	>512	>32	<=0.5	>16	<=0.5	<=2	<=1
63	-	t037	64	0.5	>4	>8	>16	2	8	>64	<=1	256	>2	>0.5	>512	>32	>4	>16	>4	<=2	<=1
73	+	t 1985	8	2	>4	>8	8	<=0.5	<=1	<=4	<=1	<=0.5	>2	<=0.016	<=64	<=4	1	>16	<=0.5	>32	<=1
75	+	t011	<=4	0.5	0.5	<=0.25	16	>4	<=1	<=4	<=1	<=0.5	>2	<=0.016	<=64	<=4	1	<=0.5	>4	4	<=1
208	+	t011	8	8	>4	>8	8	<=0.5	<=1	<=4	2	<=0.5	>2	<=0.016	128	<=4	1	>16	<=0.5	>32	<=1
316	-	t037	64	<=0.25	0.25	>8	>16	<=0.5	<=1	>64	2	<=0.5	>2	>0.5	>512	>32	<=0.5	>16	<=0.5	<=2	<=1
318	-	t037	64	<=0.25	<=0.12	>8	>16	<=0.5	<=1	>64	2	<=0.5	>2	>0.5	>512	>32	<=0.5	>16	<=0.5	<=2	<=1
		N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
		R	5	2	4	7	8	2	1	5	0	1	8	5	5	5	1	7	2	3	0
		%R	62,5	25	50	87,5	100	25	12,5	62,5	0	12,5	100	62,5	62,5	62,5	12,5	87,5	25	37,5	0
		CI	24,5-91	3,2-65	15,7-84	47,3-100	63,1-100	3,2-65	0,3-53	24,5-91	0-31	0,3-53	63,1-100	24,5-91	24,5-91	24,5-91	0,3-53	47,3-100	3,2-65	8,5-76	0-31

Figure 1. Cumulative resistance in MRSA isolated in 2014 from poultry

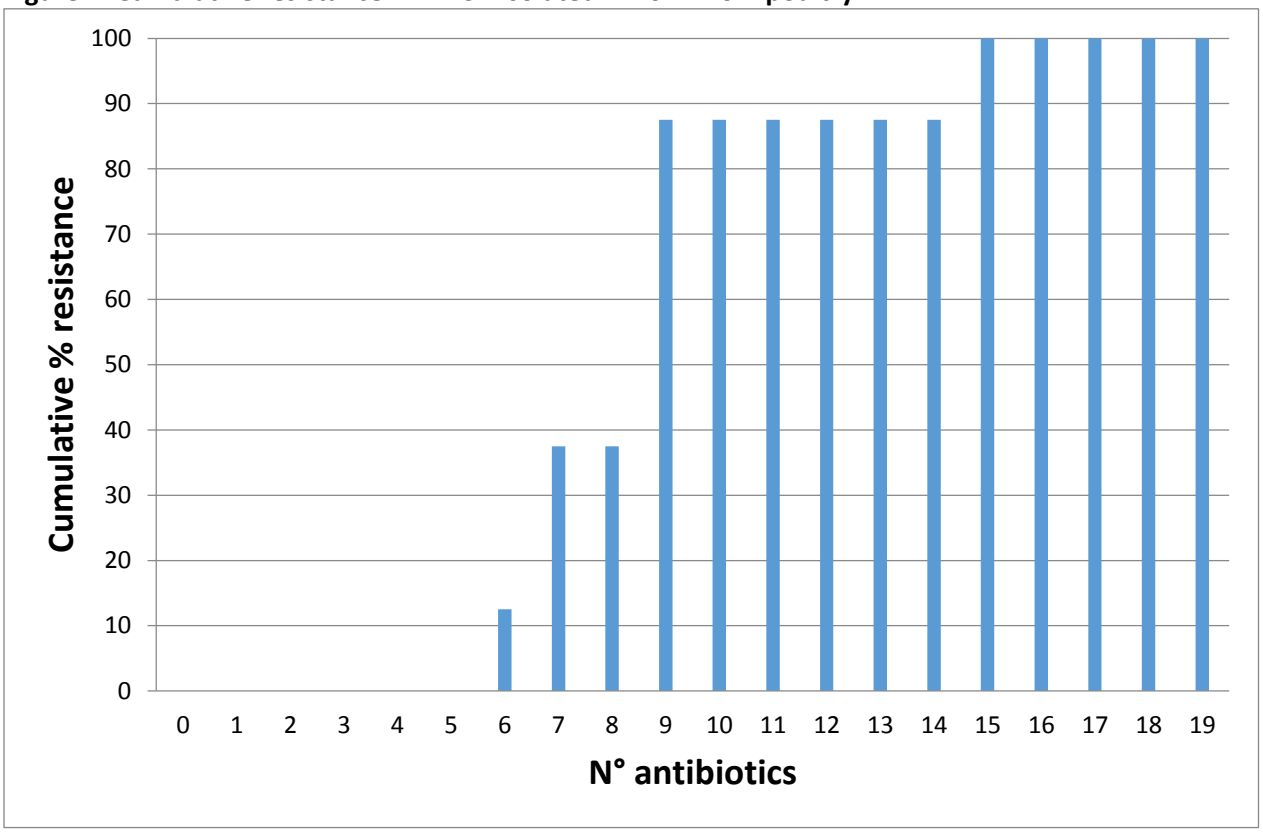
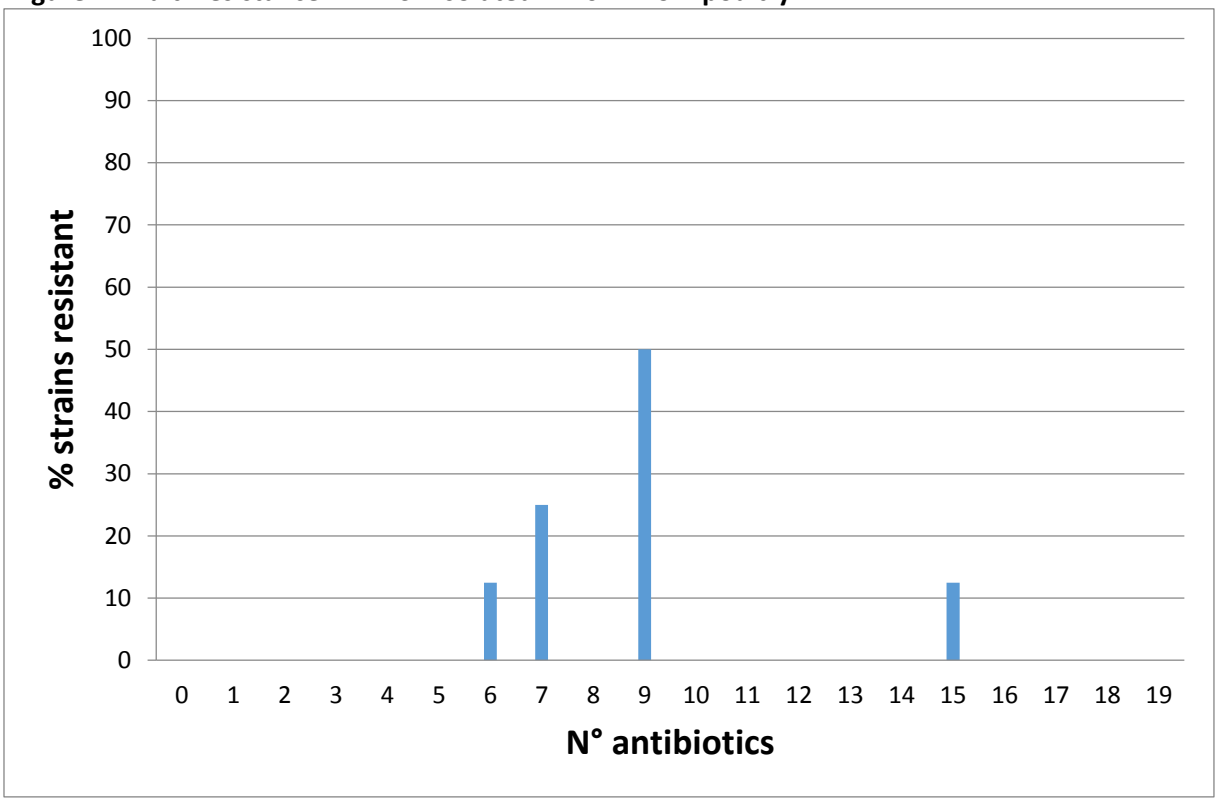


Figure 2. Multi-resistance in MRSA isolated in 2014 from poultry



5. References

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