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NEWSLETTER

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for Antimicrobial Resistance

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Content

Novel *mecA* homologue (*mecA*$_{LGA251}$) described in methicillin resistant isolates from humans and cattle

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In a recent study, performed by Holmes *et al.* isolates obtained from bovine mastitis in England were found to be phenotypically methicillin-resistant but were found negative by PCR for *mecA* and were also found negative for PBP2a by slide agglutination. However, these isolates did not show a phenotype consistent with hyperproduction of betalactamase. To investigate the genetic basis for the observed antimicrobial resistance, whole genome sequencing was performed and a divergent *mecA* homologue *mecA*$_{LGA251}$ was identified. This gene was found only 70% identical to the *mecA* gene and integrated in an SCC*mec*-like element, later denominated SCC*mec* XI. Retrospective screenings demonstrated that this gene was also present in isolates from humans in the UK and Denmark, including one Danish isolate from 1975 (García-Álvarez *et al.*, 2011; DANMAP 2010, 2011). In the positive isolates, the genetic background was CC130, CC705 and ST425 which have been previously associated with animal origin, and had not been observed in humans.

After its first description, the *mecA*$_{LGA251}$ gene has also been described in Germany in isolates belonging to CC130, isolated from infections in humans and one healthy veterinarian (Cuny *et al.*, 2011).

These findings have raised a concern regarding a possible animal origin of the isolates harbouring this gene and also regarding the need to update the methods for detection of methicillin resistance which will need to be supplemented with further testing to identify the *mecA*$_{LGA251}$. The EURL-AR will as soon as possible distribute to the NRLs the new protocols for PCR amplification of this new gene.

Further reading:

Cuny C, Layer F, Strommenger B, Witte W. Rare Occurrence of Methicillin-Resistant *Staphylococcus aureus* CC130 with a Novel *mecA* Homologue in Humans in Germany. PLoS One. 2011;6(9):e24360

DANMAP 2010, 2011
http://www.danmap.org/pdfFiles/Danmap_2010.pdf

García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA. Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis. 2011 Aug;11(8):595-603

Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2011 Aug;55(8):3765-73.

Review on methicillin-resistant *Staphylococcus pseudintermedius*

A review on methicillin-resistant *Staphylococcus pseudintermedius* has been published.

Abstract

Staphylococcus pseudintermedius is an important opportunistic pathogen of companion animals, especially dogs. Since 2006 there has been a significant emergence of methicillin-resistant *S. pseudintermedius* (MRSP) mainly due to clonal spread. This article reviews research on MRSP with a focus on occurrence, methods used for identification, risk factors for colonization and infection, zoonotic potential and control options. Potential areas for future research are also discussed.

Reference

E. van Duijkeren, B. Catry, C. Greko, M. A. Moreno, M. C. Pomba, S Pyörälä, M. Ružaukas, P. Sanders, E. J. Threlfall, J. Torren-Edo and K. Törneke. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother. Published September 19, 2011 doi:10.1093/jac/dkr367

ESBL and AmpC's in animal and food production – The EFSA BIOHAZ panel report and beyond

Hasman H; Aarestrup FM

The outcome of any investigation involving bacteria showing reduced susceptibility towards extended-spectrum beta-lactams is highly sensitive to the methods used for isolation and initial phenotypic testing. Furthermore, characterization of the bacterial isolates needs to include molecular methods to investigate both horizontal as well as vertical spread of resistance in order to determine potential epidemiological links between the isolates. And if risk factors are to be included in the investigation, then a high level of background information, such as which, how many and when antimicrobials have been used at the farm or animal level, is further required.

The EFSA BIOHAZ panel has recently published a scientific opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals[1]. The report gives a thorough introduction to the ESBL/AmpC topic as well as an in-depth presentation of the current status in relation to ESBL/AmpC beta-lactamases present among animals and food products in Europe. In addition to this, the currently available methods for isolation, detection and molecular characterization of ESBL/AmpC producing strains and their genes and plasmids are listed with relevant references to scientific papers describing the methods. Finally, recommendations and relevant risk factors involved in emergence and spread of ESBL producing bacteria are presented.

Considering the huge increase in ESBL/AmpC producing bacteria isolated from animals and food products, it becomes ever more important to be able to perform these kinds of molecular methods in a harmonized way as part of the national and EU routine surveillance programs. Especially if we want to be able to compare prevalence of resistance of if we want to be able to investigate possible spread of resistance from the animal to the human reservoir. In order to aid in this at the EU-NRL level, the EURL is hosting a laboratory course in molecular aspects of ESBL/AmpC (and MRSA) detection and characterization from 7-11 November 2011.

Further reading:

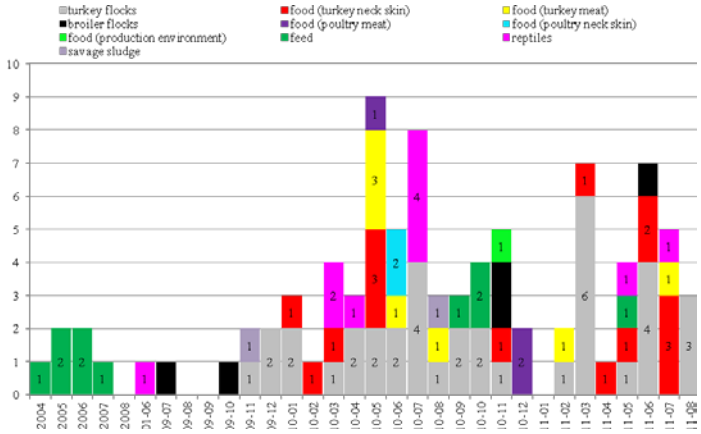
[1]EFSA opinion: Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. http://www.efsa.europa.eu/en/efsajournal/pub/2322.htm

Ciprofloxacin-resistant *Salmonella* Kentucky

Wasyl, D; Hoszowski, A; Zając, M (National Veterinary Research Institute, Puławy, Poland)

Until recently, *Salmonella* (*S.*) Kentucky was rarely observed in the National Reference Laboratory (NRL) for Salmonellosis and Antibiotic Resistance besides few feed isolates (Figure 1). Since autumn 2009, an increasing number of isolates have been referred to our laboratory for confirmatory testing. A retrospective study performed at the beginning of 2011 has identified an outbreak of *S.* Kentucky in turkey flocks without clinical symptoms, and spreading further in food production environment (turkey neck skin samples) and turkey meat. Moreover, *S.* Kentucky recovery from municipal savage sludge might suggests its further spread to human via food of animal origin [1]. An outstanding antibiotic resistance of S. Kentucky isolated in Poland was the major reason for the aforementioned study. Simultaneously, another paper on international spread of an epidemic population of *S.* Kentucky ST 198 was published [2]. The authors reported the increasing number of human cases occurring in four EU countries and the US from 2002 to 2008. They concluded that clonal spread of *S.* Kentucky was mainly related with travels to Africa and Middle East.

Figure 1: Number of *S.* Kentucky isolates by source and date of isolation



Taking the above under consideration, we intend to update the information on the occurrence of multidrug resistant *S.* Kentucky in Poland as well as to draw attention of NRLs to multi-drug resistant bacteria emerging in some EU countries.

Besides already described 51 *S.* Kentucky, during 2011 (January-September) we have received another 31 isolates from Salmonella control programmes in turkey and broiler flocks, as well as from surveillance of feed and food of animal origin. Further 10 isolates were obtained within a research project on Salmonella carriage in pet reptiles run at our laboratory. The sources and time of isolation were presented at Figure 1.

MIC testing according to EU-accepted rules (Commission Decision 407/2007/EC) was performed on 72 isolates. Table 1 shows interpretation criteria and MIC distribution of the antibiotics used.

Table 1: Minimal Inhibitory Concentration (MIC) distribution in 72 *S.* Kentucky isolates [red vertical lines demonstrate epidemiological cut-off values used for MIC interpretation]

Antimicrobials		MIC (mg/L) ≤0,008	0,016	0,032	0,064	0,125	0,25	0,5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	AMP							5	5	1					61					
Ceftazidime	CAZ						3	59	9				1							
Cefotaxime	CTX				7	57	7					1								
Gentamicin	GEN						1	5	5	1			5	45	10					
Kanamycin	KAN										62	7					2			
Streptomycin	STR									1	1	8	4	36	20	2				
Nalidixic acid	NAL										7	1				64				
Ciprofloxacin	CIP			3	4			1					39	24						
Sulfamethoxazole	SMX												1	1	6	2	2			60
Trimethoprim	TMP							71			1									
Colistin	COL									69	3									
Chloramphenicol	CHL									8	60	3				1				
Florfenicol	FLR									24	44	3				1				
Tetracycline	TCY								9	3			1	17	38	4				

The percentage of strains with possible resistance mechanism (NWT – non wild type) was shown in Figure 2 whereas the observed resistance profiles were listed in Table 2. The most frequent resistance profile, comprising seven compounds from five antimicrobial classes, was observed in 68.1% of tested isolates. The most complex profile, including cephalosporin resistance, have not been found in any other *S.* Kentucky but the previously described strain producing CTX-M [1]. Multidrug resistance (more than 3 antimicrobial classes) was observed in 87.5% of isolates.

Microbiological resistance to quinolones (both Nal and Cip),

the second critically important antibiotic class, was observed in 88.9% of tested isolates. All but one NWT isolates showed extremely high MIC value of ≥8 mg/L. This finding is in accordance to the results of Le Hello *et al*. [2], who had identified three mutations in *gyrA* and *parC* as a genetic background of the resistance. We would like to emphasize, that MICCip ≥ 8 has been observed only in 4 (0.13%) out of approximately 3400 Salmonella strains tested in our lab with microbroth dilution method over the last eight years. Those strains belonged to *S.* Enteritidis and *S.* Newport. Quinolone WT strains originated mostly form feed, feed components, and pet reptiles (respectively, three and four isolates). Interestingly, three isolates from carnivore reptiles from two distinct households showed MICCip ≥ 8 mg/L. It suggests that those animals might be infected by the epidemic strains observed in turkey production [1] and humans [2]. The hypothesis is currently under investigation with PFGE typing.

Although there is no direct evidence, we assume that it might be an epidemiological link between described isolated of human and animal origin. To reveal this, we are sequencing Quinolone Resistance Determining Regions of gyrase and topoisomerase IV genes to define quinolone resistance mechanism. The presence of plasmid mediated resistance genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* and *aac*(6')-Ib-cr) are also under investigation. We have already confirmed (*S.* Le Hello, personal communication) that the most frequent PFGE profile found in our study [1] is indistinguishable from the PFGE profile of *S.* Kentucky ST198 [2].

Figure 2: Percentage of non-wild type *S.* Kentucky isolates

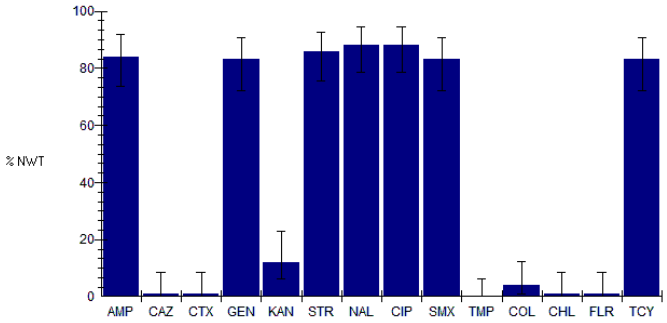


Table 2: Resistance profiles observed in *S.* Kentucky

R-profile	No of isolates
AmpCtxCazGenKanStrNalCipSmxTcy	1
AmpGenKanStrNalCipSmxChlFlrTcy	1
AmpGenKanStrNalCipSmxTcy	4
AmpGenStrNalCipSmxColTcy	3
AmpGenStrNalCipSmxTcy	49
AmpKanNalCip	2
AmpKanStrNalCip	1
GenStrNalCipSmxTcy	2
NalCip	1
Str	1
sensitive	7

The primary infection source remains unknown. However, there are only few turkey breeding farms in Poland and most of the fattening flocks are imported as hatching eggs or 1-day old poults. Based on that we might presume that primary infection source was outside Poland. This is in agreement with Le Hello *et al.* [2] who draw back the evolution scenario of multi-drug resistant *S.* Kentucky throughout Africa and indicate such plausible sources of infection as chicken and turkey production, aquatic environment contaminated by humans or poultry. From there the clone spread through travellers to the EU and North America. Finally, the serovar has reached also Polish turkey production sector.

With this note we aim to alert the network of national reference laboratories to the emerging MDR *S.* Kentucky, which occurrence may not be limited to few countries mentioned in the citations.

Further reading:

Wasyl, D., A. Hoszowski, First isolation of ESBL-producing Salmonella and emergence of multiresistant *Salmonella* Kentucky in turkey in Poland. Food Research International, 2011. DOI: 10.1016/j.foodres.2011.07.024

Le Hello, S., Hendriksen, R.S., Doublet, B., Fisher, I., Nielsen, E.M., Whichard, J.M., Bouchrif, B., Fashae, K., Granier, S.A., Jourdan-Da Silva, N., Cloeckaert, A., Threlfall, E.J., Angulo, F.J., Aarestrup, F.M., Wain, J., Weill, F.X., International Spread of an Epidemic Population of Salmonella enterica Serotype Kentucky ST198 Resistant to Ciprofloxacin. The Journal of infectious diseases, 2011, DOI: 10.1093/infdis/jir409

Characterization of *qnr*-positive *E. coli* isolates from food-producing animals

A letter on 'Characterization of *qnr*-positive *Escherichia coli* isolates from food-producing animals in the Netherlands' by Veldman K, van Essen-Zandbergen A, Kant A, Mevius D. is available in the J Antimicrob Chemother. (2011)

The study demonstrates the presence of *qnr*-genes on two different types of plasmids (IncX2 and non-typeable) in *E. coli* isolated from animals (veal calves and poultry chicken). Moreover, it describes the co-existence of three different extended beta-lactamase genes on three different plasmids in a single *E. coli* isolate harbouring qnrS1.