National Food Institute
Technical University of Denmark

NEWSLETTER

to the
National Reference Laboratories for Antimicrobial Resistance

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Looking forward to working with you!

With much anticipation we – the Community Reference Laboratory for Antimicrobial Resistance – have commenced coordinating this network of sharing experience and knowledge about activities concerning antimicrobial resistance. With this newsletter we will draw your attention to recent developments concerning three very relevant subjects: MRSA, quinolone resistance and breakpoints.

Methicillin-resistant *Staphylococcus aureus* in animals

By Frank M. Aarestrup

From being almost exclusively a nosocomial pathogen Methicillin-resistant *Staphylococcus aureus* (MRSA) have during the last two decades emerged into the community and have recently also caused infections in and colonized pets and production animals. MRSA have been detected in cattle, chickens, horses, pigs, dogs, rabbits, seals, birds and cats. The colonization in animals has in several cases been implicated in infections in humans and MRSA should today be considered a zoonosis. It is however, important to distinguish between the epidemiology of MRSA in relation to production animals, where a new clone seemingly is emerging, and pet animals, that are infected with classical human variants of MRSA.

Production animals

In relation to colonisation in pigs, MRSA were in October 2004 isolated from a young mother with mastitis in the Netherlands. The father and daughter were also found to carry MRSA. Six months later the daughter was admitted to a hospital for surgery and the entire family was again found positive for MRSA. Normally, the Dutch population have a <1% MRSA incidence, commonly associated with treatment in foreign hospitals. As the father was a pig farmer the finding initiated a number of studies. In a small survey of pig farmers, MRSA was found in 23% (6/26 farmers) and in another survey of veterinarians and veterinary students in the Netherlands that found an average of 4.6% were carriers of MRSA. A study in the pig population has revealed a colonisation rate of 40% of all slaughter pigs and 80% of pig slaughter batches (out of a total of 54 batches and a total of 540 animals examined) in the Netherlands. All isolates belong to a specific clone ST398, which seems to have established itself in the pig population in the Netherlands from where it transfers to humans. This clone was recently isolated from skin infection in a pig and also from a dairy cattle farm in the Netherlands from cows suffering from mastitis caused by this strain.

The same clone (ST398) of MRSA was in the fall 2006 detected in patients in Denmark, most of which have had close contacts to production animals, mainly swine. In addition, a single MRSA isolate has also been found in a swine farm in Denmark. Studies are currently being conducted into the occurrence of MRSA among production animals in Denmark. Information from other countries has at this time not

been available for us. This sequence type has also been described in strains isolated from pigs and farmers in France, though the French isolate from a pig was not methicillin resistant. MRSA ST398 have also been described in Germany, from four veterinarians (nasal carriage), a dog with a skin/wound infection and a foal with sinusitis at one veterinary centre and from a single pig sampled at a veterinary school and found to be colonised. Two Austrian horses with wound infections were also found to be colonised with MRSA ST398, though these isolates differed at the molecular level from the German isolates and were thought to be unrelated. Eleven other human MRSA ST398 isolates have been detected in Germany, including seven from cases of ventilator-associated pneumonia.
With our current knowledge it seems quite evident that ST398 is a MRSA clone transmitted from pigs to humans; its origin is unknown, though it seems probable that it – or its antecedents - will have originated in humans. Further studies are underway in several countries, but it seems likely that MRSA ST398 are widespread in the pig populations, in at least the Netherlands and Denmark, but most likely in all European countries with intensive swine production. ST398 is mainly found to colonise animals, but have in a few cases been found to cause infections. The limited number of reports is probably due to the difficulties of isolating this bacterium from animals because it is necessary to use selective enrichment. It must be expected that several new reports will be published in the near future. The reason for the colonization of MRSA ST398 in pigs or the epidemiology of this clone is currently not known; it possibly first emerged in 2003, as it was not detected in 2002 in the human monitoring being done in Holland, or in monitoring from 1992-2003 of human isolates in Germany. It can be speculated that the use of cephalosporins and other antibiotics have provided a niche for this clone, but until further studies are carried out this is merely speculation.

Pet animals

MRSA have also been found in pet animals such as dogs, cats and horses. In several cases the bacteria have caused infections in the animals that have been difficult to treat because of the multiple resistance. The MRSA has been of the classical human types and the initial spread were most likely from humans to the animal. Cases have been described where such animals colonised with MRSA have acted as vectors for the spread of MRSA to other humans.

Comments and recommendation

MRSA should based on our current knowledge be considered a zoonosis. Pet animals can act as a reservoir for the bacterium from where it can transfer to and cause infections in humans. In infection control pet animals should probably be treated as any other family member. In production animals the situation is different and still somewhat unclear. It seems like this is a single clone that might have adapted itself to colonise animals (pigs and perhaps cattle) from where it can spread to humans. The importance for human health and the possibilities for infection control are currently unclear. In the Netherlands it is advised to keep pig breeders, if they are admitted to a hospital, in isolation until surveillance cultures are proven negative. This also applies to veterinarians and slaughterhouse personnel. For cattle breeders screening without isolation on admission to a hospital is sufficient. Further studies providing new information will become available in the near future.

Diagnostic laboratories should be aware that MRSA might be isolated from animals. Whenever, any suspicion arises, the isolates should be send to a reference laboratory, for example the Community Reference Laboratory, for verification. If possible surveys using selective enrichment procedures should be conducted. The CRL would be happy to advice on the planning of such surveys. We kindly ask you to inform the CRL if you find MRSA in production animals in your country.

Suggested further reading

Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, Voss A, Wannet WJ, de Neeling AJ. 2006. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob 10;5:26.

Leonard FC, Markey BK. 2007. Meticillin-resistant *Staphylococcus aureus* in animals: A review. Vet J. Jan 8; [Epub ahead of print].

Witte W, Strommenger B, Stanek S, Cuny C. 2007. Methicillin-resistant *Staphylococcus aureus* ST398 in Humans and Animals, Central Europe. Emerg Infect Dis 13:255-8.

Transferable low-level resistance fluoroquinolone resistance in *Enterobacteriaceae* - recent developments

By Frank M. Aarestrup

Until recently, chromosomal mutations in different genes involved in DNA-transcription and replication were considered the main mechanisms of quinolone resistance in *Enterobacteriaceae*. A new and transferable mechanism was described in 1998 in a *Klebsiella pneumoniae* isolate obtained from a patient in 1994 in Alabama, USA. This mechanism named *qnrA* encodes a protein that blocks the action of fluoroquinolones. Since then two other *qnr*-genes (*qnrB* and *qnrS*) have been identified. The encoded QNR proteins are not only able to protect the gyrase and reduce its susceptibility to fluoroquinolones, but also importantly increase the frequency of mutants. Plasmid mediated quinolone resistance was originally found very rarely, but seems to have spread more rapidly than expected and is now found in the US, Africa, Asia and also in Europe. The genes are often located on transferable plasmids together with other resistance genes especially genes encoding resistance to cephalosporins. The genes have been detected in several species including *Salmonella*.
Presence of the *qnr* genes alone does not necessarily mediate full resistance to nalidixic acid and thus, makes it uncertain to use nalidixic acid for screening for fluoroquinolone resistance. This is in contrast to the mutation-mediated resistance where one mutation encodes low-level resistance to fluoroquinolones and full resistance to nalidixic acid. Low-level fluoroquinolone resistance is difficult to detect in routine diagnostic laboratories and these isolates might easily be considered susceptible especially when using diffusion testing.

In 2006 another mechanism of transferable quinolone resistance was reported. The *cr* variant of *aac(6')Ib* encodes an aminoglycoside acetyltransferase that confers resistance to ciprofloxacin by N-acetylation of its piperazinyl amine. This variant has two amino acid changes W102R and D179Y, which together enable this aminoglycoside resistance mechanism to also modify ciprofloxacin. This mechanism was described in *E. coli* isolates from Shanghai, but has since been found with a high prevalence in the United States among *Enterobacteriaceae* strains with ciprofloxacin MIC ≥0,25 and reduced susceptibility to ceftazidime. It has, furthermore, been described in Portugal in a gene cassette including the OXA –1 gene in CTX-M-15 and TEM-1 positive strains, in CTX-M positive *K. pneumoniae* from Nigeria, and in *E. coli* from the United Kingdom and Denmark. As for *qnr* this new fluoroquinolone resistance mechanism seem to be located on multiple resistance plasmids which commonly also encode cephalosporin resistance. This new mechanism is seemingly very common and the limited number of reports so far is probably only due to the fact that this gene was discovered very recently. This gene has also been found in isolates in combination with chromosomal mutations and in isolates with *qnr* genes. *aac(6')Ib-cr* is seemingly not active against enrofloxacin, but its activity against other veterinary fluoroquinolones and nalidixic acid is currently not known.

Comments and recommendations

Several new mechanisms of transferable quinolone resistance have been detected recently. These mechanisms are seemingly more widespread than expected and worryingly very often located on multiple resistance plasmids often in combination with genes encoding resistance to cephalosporin. Thus, the use of fluoroquinolones might now not only select for quinolone resistant clones but also might select for transferable resistance to both fluoroquinolones and cephalosporins. These two antimicrobial classes are normally the drugs of choice for treatment of *Salmonella* infections in humans. These mechanisms mediate low-level resistance to fluoroquinolones but not necessarily to nalidixic acid and might therefore be very difficult to detect in diagnostic laboratories. It is recommended that low-level break points for fluoroquinolones (MIC ≤ 0.06 mg/L for ciprofloxacin) are used for the detection of fluoroquinolone resistance. Whenever there is doubt, please send the isolates to the Community Reference Laboratory for verification.

Suggested further reading

Hopkins KL, Davies RH, Threlfall EJ. 2005. Mechanisms of quinolone resistance in Escherichia coli and Salmonella: recent developments. Int J Antimicrob Agents. 25: 358-73.

Li XZ. 2005. Quinolone resistance in bacteria: emphasis on plasmid-mediated mechanisms. Int J Antimicrob Agents 25: 453-63.

Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, Bush K, Hooper DC. 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med. 12: 83-8.

Robicsek A, Jacoby GA, Hooper DC. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis. 6: 629-40.

Breakpoints

By Frank M. Aarestrup and Dik J. Mevius

The European Food Safety Authority (EFSA) has established a working group on developing harmonized schemes for monitoring antimicrobial resistance in zoonotic agents. This working group has provided a list of antimicrobials to be included in the antimicrobial resistance monitoring for each zoonotic agent, the epidemiological cut-off value for each antimicrobial to be used to determine susceptibility, and the advised concentration range to be tested for each antimicrobial. The list below is from their work, which has been approved by the EFSA task force. After minor corrections the report of the working group will soon be available on the EFSA website (www.efsa.com). The antimicrobials listed represent the minimum requirements for Member States to include in their test panels. For these antimicrobials the epidemiological cut off values are mandatory. The concentration ranges are advised to be used in dilution tests for optimum detection of acquired resistance. The purpose behind this list is to optimise the harmonisation in MIC results between Member States.

	Antimicrobial	Cut-off value (mg/L) R>	Concentration range to be tested (mg/L)
Salmonella	Cefotaxime	0.5	0.06 – 8
	Nalidixic acid	16	2 – 256
	Ciprofloxacin	0.06	0.008 – 8
	Ampicillin	4	0.5 – 64
	Tetracycline	8	0.5 – 64
	Chloramphenicol	16	2 – 256
	Gentamicin	2	0.25 – 32
	Streptomycin*	32	2 – 256
	Trimethoprim	2	0.25 – 32
	Sulphonamides**	256	8 – 1024
Campylobacter jejuni	Erythromycin	4	0.5 – 64
	Ciprofloxacin	1	0.06 – 8
	Tetracycline	2	0.125 – 16
	Streptomycin	2	0.5 – 32
	Gentamicin	1	0.125 –16
Campylobacter coli	Erythromycin	16	0.5 – 64
	Ciprofloxacin	1	0.06 – 8
	Tetracycline	2	0.125 – 16
	Streptomycin	4	0.5 – 32
	Gentamicin	2	0.125 –16

* Breakpoint advised by ARBAO-II, ** CLSI breakpoint

Coming CRL-activities

- EQAS 2006 – you will receive a report describing and evaluating the results from the EQAS
- Questionnaire – as a means of collecting information from the NRL's on the activities in relation to antimicrobial resistance, you will be asked to fill in a questionnaire. The questionnaire will be sent to you in March and will include questions on monitoring programmes, on antimicrobial agents, ranges and methodologies as well as on needs for training and protocols
- Workshop – on May 3rd-4th you are invited to a workshop in Copenhagen. A programme will be sent to you shortly

See you soon!

In a few months we will be meeting for the workshop where we, among other things, will be discussing the subjects mentioned in this newsletter. The network now consists of 29 laboratories in 26 countries in the EU, and we expect these numbers to rise since a few Member States have not yet designated their NRL.