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Time of the visit: 2.5-7.5.2010
Host institution: University Hospital of North Norway
National Reference Centre for Detection of Antimicrobial Resistance
Tromsø, Norway
Purpose of the training: To learn S1 nuclease PFGE, hybridization and detection

In VRE strains the location of *vanA* and *vanB* resistance genes can reside in chromosome, plasmid or both. As the size of enterococci plasmids can be of high-molecular-weight their purification from the chromosomal DNA is technically demanding. The article by Barton describes a general method for detection and sizing of large plasmids avoiding the plasmid purification. In the protocol S1 nuclease nicked linear plasmids are separated inside agarose plugs by pulsed-field gel electrophoresis (PFGE) (Barton *et al.*, 1995). The separated plasmids can be transferred to nylon membranes and hybridized with DNA probe targeted to gene under investigation. This method has been successfully used for both gram-negative and gram-positive bacteria to study the chromosomal or plasmid origin of different genes (Biavasco *et al.*, 2007; Samuelsen *et al.*, 2009).

The purpose of my training was to gain technical knowledge and assistance for the method described above.

My hosts for the one week visit to Norway's National Reference Centre for Detection of Antimicrobial Resistance in University Hospital of North Norway were Professor Arnfinn Sundsfjord and Dr. Kristin Hegstad. The person responsible for the practical training was Torill Solvær Rosvoll and she had scheduled the week to work with the protocol. We performed the PFGE protocol with ten Finnish *E. faecium* strains with verified resistance genes (either *vanA* or *vanB*) which had been sent to the laboratory in advance. The used S1 nuclease concentration and the PFGE running conditions were planned out to be the best possible. We also made two parallel gels at the same time for two different blotting systems: Vacuum blotting and Turbo blotting. After the PFGE runs, we could, however, see that the conditions had not been optimal and the

plasmids smaller than 20 kb could not be detected. As the purpose was to locate the *van* genes which usually reside in the larger plasmids and to learn the protocol the work was continued.

The PFGE gels were blotted with two different systems as described above. We also restained the gels after blotting to evaluate the transfer efficiency which seemed to be good with both systems. The hybridization was done with a *vanB* probe with DIG-labeling and we made the probe with primers designed to recognize all *vanB*-gene types. A dilution series of the probe and a control DNA of known concentration were spotted into a membrane and the concentration of the probe was estimated.

Unfortunately, the films were blank after the detection. The reason for that remained unclear; at least the pictures of PFGE gels were considered as normal and the probe had a good concentration. The next step in my work is to set up the system in our laboratory in Finland, to repeat the above mentioned experiments and to start analyzing a larger set of Finnish VRE strains.

I would like to thank the ESCMID for the grant that made this trip possible and gave me the opportunity to learn this laboratory technique. Also I want to say a warm thank you to my hosts and the laboratory and research staff in Tromsø who made me feel welcome, taught me and answered to my continuous questions.

Barton, B. M., Harding, G. P. & Zuccarelli, A. J. (1995). A general method for detecting and sizing large plasmids. *Anal Biochem* **226**, 235-240.

Biavasco, F., Foglia, G., Paoletti, C. & other authors (2007). VanA-type enterococci from humans, animals, and food: species distribution, population structure, Tn1546 typing and location, and virulence determinants. *Appl Environ Microbiol* **73**, 3307-3319.

Samuelsen, O., Naseer, U., Tofteland, S., Skutlaberg, D. H., Onken, A., Hjetland, R., Sundsfjord, A. & Giske, C. G. (2009). Emergence of clonally related Klebsiella pneumoniae isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden. *J Antimicrob Chemother* **63**, 654-658.