DANSK SELSKAB FOR KLINISK MIKROBIOLOGI

Yngre Kliniske Mikrobiologers temadag om Klinisk Mikrobiologisk forskning

27. oktober 2017 på Odense Universitetshospital
Klinisk Mikrobiologisk Afdeling. JB: Winsløvsvej 21.2 Sal. Store bibliotek.

Program

09.45 Ankomst, registrering og kaffe

10.15 Velkomst ved Thomas Vognbjerg Sydenham, Formand for YKM

10.20 Epidemiologisk forskning i Klinisk Mikrobiologi
15 min indlæg (10min oplæg + 5min til spørgsmål), Chair: Frederik Bötius Hertz
1. Tick-borne Encephalitis virus in Denmark
   v. Nanna S. Andersen
2. Community-acquired *Escherichia coli* bacteremia and risk of cancer diagnosis
   v. Kirstine K. Søgaard
3. S. *maltophilia* Bloodstream Infections Caused by Closely Related Strains,
   v. Sofie Skovmand Rasmussen

11.05 Pause

11.30 Forskning i laboratoriemetoder og diagnostik i Klinisk Mikrobiologi
15 min indlæg (10min oplæg + 5min til spørgsmål), Chair: Nanna S. Andersen
1. Predicting ß-lactam susceptibility from the genome of *Streptococcus pneumoniae, Streptococcus pseudopneumoniae* and *Streptococcus mitis group* isolates
   v. Helle Brandt Eriksen
2. Reproducerbarhed af urindyrkninger i et Klinisk Mikrobiologisk laboratorium
   v. Kristina Træholt Franck
3. Impact of bead beating or heat incubation prior to DNA extraction on diagnostic microbiome results from bronchoalveolar lavage samples – a pilot study
   v. Thomas Vognbjerg Sydenham

12.15 Frokost

13.15 Klinisk Mikrobiologisk forskning nu og i fremtiden, Chair: Hans Linde Nielsen
1. Som **Klinisk Mikrobiolog** ved Professor Henrik Schønheyder.
2. Som **Kliniker** ved Professor og Ledende Overlæge Henrik Nielsen.
3. Som **Leder** ved Ledende Overlæge Christian Østergaard.

15.15 Pause

15.30 **Workshops**, enten

1) Data med R - Hvorfor? En blid introduktion /Marc Trunjer Kusk Nielsen

2) Introduktion til forskning
   a) Fra idé til projekt - erfaringer fra et laboratoriebaseret projekt
      v. Lise Tornvig Erikstrup
   b) Data tilladelser v. Nanna Skaarup Andersen
   c) Funding og skriveprocessen v. Kirstine Kobberøe Søgaard

17.00 Opsamling ved Thomas Vognbjerg Sydenham, og udpegelse af arrangører i 2018

18.00 Middag på Restaurant Flammen, Kongensgade, Odense (egenbetaling og frivilligt, naturligvis!)

Vel mødt. På vegne af arrangørgruppen

Thomas Vognbjerg Sydenham, Formand for Yngre Kliniske Mikrobiologer
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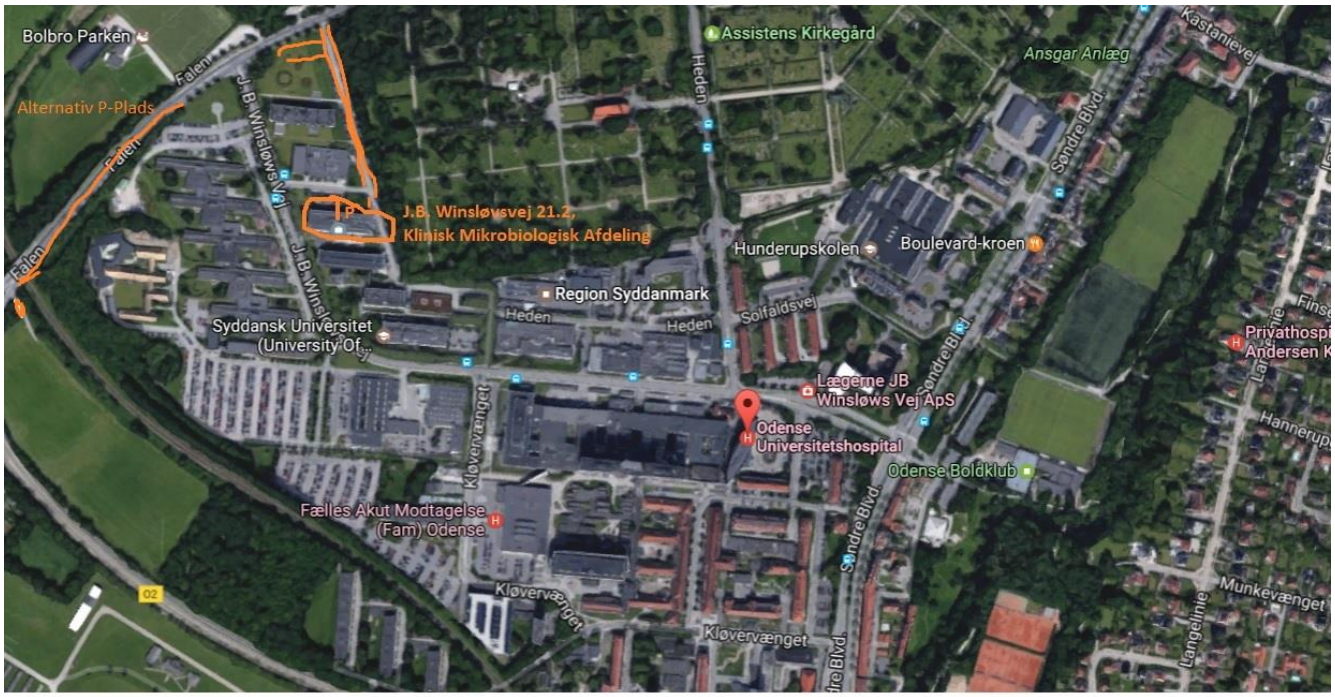
Parkering:
Det er muligt at parkerer ved KMA, såfremt man kommer i bil.
Det er dog meget svært at få en P-plads efter kl. 8.
Lykkedes det at få en P-plads (markeret med orange), da kan man registrere sin bils nummerplade på IPAD'en, der hænger i stueetagen i WP 21.1. Det giver tilladelse til parkering hele dagen. Der er enkelt 2-timers parkeringspladser

Alternativt kan man forsøge at finde en P-plads på Falen.



Tick-borne infectious in Denmark

Authors: Andersen NS[1,2], Jensen PM[3] Kolmos HJ[1] and Skarphédinsson S[2].
1: Research Unit of Clinical Microbiology, Institute of Clinical Research, Faculty of Health Science, University of Southern Denmark.
2: CCEVI - Clinical Center of Emerging and Vector-borne Infections, Odense University Hospital, Denmark.
3: Department of Plant- and Environmental Sciences, University of Copenhagen.

Introduction: *Ixodes ricinu*s ticks are known to be the vector of many human pathogens. But the prevalence of the tick-borne pathogens in the ticks varies greatly both with regard to the different pathogens and regionally. In Denmark previous nationwide studies have found that *Borrelia burgdorferi* is the tick-borne pathogen most widely dispersed in ticks with a prevalence of between 5-15% (1,2). It is also the most common tick-borne disease with a yearly incidence of 10/100.000 suffering from neuroborreliosis alone (3). While monitoring borreliosis risk by monitoring *Borrelia* tick burden may be feasible, this is more difficult with regard to other infections with lower prevalence or more marked regional variation in distribution. A good example of this is tickborne encephalitis virus (TBEV), known for its focal distribution and low prevalence in ticks(<1%) in endemic areas(4). In recent years an increase in TBE cases and change in distribution has made the need for better surveillance clear.

In Denmark, TBE cases have so far only been identified from 2 areas, Bornholm and Tokkekøb hegn in North Zealand. Previous sentinel studies have indicated that TBEV exists outside the known endemic areas (5,6). To explore this further, we performed 3 projects):
1) A high intensity sero-prevalence study of TBE-virus complex throughout Denmark using roe deer (*C. capreolus*) as sentinels (7).
2) Since the TBE-virus complex consists of several viruses including TBEV and Louping-ill virus (LIV) and both have been found in Denmark we wanted to determine whether there in fact was TBEV or LIV in the ticks in the areas that had yielded TBE-virus complex sero-positive roe deer (4).
3) In the last project we wanted to determine the seroprevalence of TBE-complex and *Borrelia burgdorferi s.l.* in a population at risk.

Method:
1) TBE-complex in roe deer: 804 blood samples from roe deer were examined for the existence of specific antibodies against the TBE-virus complex by neutralisation-test (NT).
2) TBE-complex in ticks: Ticks were collected from 6 locations outside the previously known endemic areas based on positive TBE-virus complex NT-titer (1:40 and above, the highest being 1:960) in the roe deer samples.
3) Tick-borne infections in a population at risk: Hunters, forest workers, and people with frequent recreational activities in nature were invited to participate in this study, supplying a blood sample and afterwards answering an electronic survey on i.e. vector exposure, vaccination status and whether they had fallen ill after a tick-, fly- or mosquito bite.
Serum was tested for IgG antibodies against *B.b.s.l.* and TBEV by ELISA tests (Enzygnost® Lyme link VlsE/IgG and Anti-TBE/FSME Virus (IgG, IgM) on the BEP2000 system, Siemens Healthcare A/S).

Results:
1) TBE-complex in roe deer:
51 samples were found positive. Positive samples were found clustered in 19 new municipalities across the whole country and 7 municipalities coincided with previous studies.

2) TBE-complex in ticks:
7538 ticks were divided into 980 pools. Only two pools yielded TBE-positive ticks, both were found on the island of Bornholm

3) Tick-borne infections in a population at risk:
591 participants were included in the study, of these 340 completed the survey, of these 310 are men. The results of these 340 are presented.

Ilness after tickbite: 13.9 % (45/324) reported illness after a tickbite. 16 did not answer. 42 of these contacted their GP and 7 were hospitalized of whom 3 were diagnosed with Lyme neuroborreliosis, 1 with Murine typhus, 1 with viral meningitis and the last two were never diagnosed.

Borrelia burgdorferi: Of these 45 only 13 had antibodies against *B.b.*s.l. and 1 had antibodies against TBE, 4 had antibodies against both TBE and *B.b.*s.l. This results in 68.8 % (31/45) reporting illness without detectable antibodies 21,47 % (73/340) had IgG antibodies against *B.b.*s.l., 2.65 % (9/340) were inconclusive and 75.88 % (258/340) negative. But only 17.8 % (13/73) reported iless after tickbite. There are significantly more participants above 50 years of age with IgG antibodies against *B.b.*s.l. (P = 0,04).

TBE antibodies: 10,59 % (36/340) had IgG antibodies against TBE, 2.06 % (7/340) were inconclusive and 87.35 %

(297/340) negative. Of the 36 positve participants only one had not been vaccinated against any flavivirus. The same were true for 2 with inconclusive results. 49 participants were vaccinated against TBE, but only 32 (65.31 %) had antibodies against TBE.

Discussion: Using roe deer as sentinel it seems there is viruses within the TBE-complex circulating in Denmark. When trying to verify the specific virus, ticks is not well suited due to the low prevalence even in endemic areas. When looking at the population at risk it seems the seroprevalence of antibodies against TBE IgG not related to TBE-vaccination is very limited. Therefore, it seems TBE is not a big problem in Denmark, but we still recommend monitoring of the pathogen in Denmark, and to consider TBE as a differential diagnosis in patients with compatible illness.

Refrences:

1. Jensen PM, Hansen H, Frandsen F. Spatial risk assessment for Lyme borreliosis in Denmark. Scand J Infect Dis. 2000;32(5):545–50.
2. Stensvold CR, Al Marai D, Andersen LO, Krogfelt KA, Jensen JS, Larsen KS, et al. Babesia spp. and other pathogens in ticks recovered from domestic dogs in Denmark. Parasit Vectors [Internet]. 2015 Dec [cited 2015 Jun 2];8(1). Available from: http://www.parasitesandvectors.com/content/8/1/262
3. Dessau RB, Espenhain L, Mølbak K, Krause TG, Voldstedlund M. Surveillance and outbreak reports: Improving national surveillance of Lyme neuroborreliosis in Denmark through electronic reporting of specific antibody index testing from 2010 to 2012. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull [Internet]. 2015 Jun 16 [cited 2015 Aug 4];20(28). Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21184
4. Jensen PM, Skarphedinsson S, Semenov A. [Densities of the tick (Ixodes ricinus) and coexistence of the Louping ill virus and tick borne encephalitis on the island of Bornholm]. Ugeskr Laeger. 2004 Jun 21;166(26-31):2563–5.
5. Skarphedinsson S, Jensen PM, Kristiansen K. Survey of tickborne infections in Denmark. Emerg Infect Dis. 2005 Jul;11(7):1055–61.
6. Lindhe KES, Meldgaard DS, Jensen PM, Houser GA, Berendt M. Prevalence of tick-borne encephalitis virus antibodies in dogs from Denmark. Acta Vet Scand. 2009;51.
7. Nanna Skaarup Andersen, Sigurdur Skarphedinsson, Per Moestrup Jensen, Carsten Riis Olesen, Hens Jørn Kolmos. Nationwide seroprevalence study of Tick-borne encephalitis virus in Danish roe deer (C. capreolus). In: ICLB 2015. Vienna, Austria; 2015.

Stenotrophomonas maltophilia Bloodstream Infections Caused by Closely Related Strains

Introduction

Stenotrophomonas maltophilia (*S. maltophilia*) is generally considered a bacterium of low pathogenic potential but in patients with compromised immunity and/or prolonged antibiotic treatment, it can cause bacteremia associated with high mortality.

The aim of this study was to explore the genetic relatedness of *S. maltophilia* in blood cultures from high risk patients, assessing whether bacteremia is caused by sporadically occurring strains or a limited number of bacterial strains that may persist in the hospital environment.

Method

In the period 2010-2016, 41 patients at Odense University Hospital had bacteremia with *S. maltophilia*. For this study, we included isolates from all patients hospitalized at intensive care units, pediatric wards, and departments of hematology, and in addition two epidemiologically unrelated isolates. Twenty-eight blood isolates of *S. maltophilia* from as many patients collected between 2010 and 2016 were available for whole genome sequencing (WGS). For each isolate, the sampling date and hospital ward was noted. The isolates were sequenced by 2 x 150 bp paired ends sequencing at a MiSeq instrument. Multi locus sequence typing was done by extracting sequence types (STs) by MLST 1.8 (cge.cbs.dtu.dk/services/MLST/) from genome sequences assembled by SPAdes 3.9 (cge.cbs.dtu.dk/services/SPAdes/) and SNP-based phylogenetic trees were constructed from the read files using CSI Phylogeny 1.4 (cge.cbs.dtu.dk/services/CSIPhylogeny/).

Results

Twelve isolates belonged to five different clusters representing five sequence types (STs). Within these clusters, the SNP-based phylogenetic analysis showed that the genomes differed by ten or less SNPs. The remaining sixteen isolates differed to any other isolates by at least 300 SNPs and were either untypable or belonged to STs only found once.

Among the five STs that were represented by more than one isolate, ST-39 was isolated from two patients in pediatric wards in 2011 and 2012, and ST-26 from two patients admitted to two separate intensive care units in 2012 and 2014. Three isolates belonging to ST-77 were isolated from two patients in the intensive care unit in 2012 and 2013 and from a patient in the hematology ward in 2016. ST-94 was from one patient in the hematology ward and one pediatric patient, both from 2013. ST-29 included three isolates from 2012, 2015, and 2016, from two patients admitted to the hematology ward and one patient in the intensive care unit.

Conclusion

Among 28 bloodstream isolates of *S. maltophilia* from hospitalized patients, we identified 12 isolates that were closely related (ten SNPs or less) to at least one other isolate. These isolates appeared in patients from different departments with long time intervals.

The findings suggest that some bloodstream infections with *S. maltophilia* in our hospital may be caused by strains that survive and spread within the local hospital environment for several years. This calls for further investigations into the source of acquisition and modes of transmission in order to prevent nosocomial infections with this pathogen.

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Abstract

Predicting β-lactam susceptibility from the genome of *Streptococcus pneumoniae, Streptococcus pseudopneumoniae* and *Streptococcus mitis group* isolates

Helle Brander Eriksen, Kurt Fuursted, Kristian Schønning, Hans-Christian Slotved

The proportion of *Streptococcus pneumoniae (Sp)* isolates with decreased susceptibility towards penicillin is low in Denmark (6.2%), but high in other parts of Europe (24-39%). Decreased susceptibility occurs due to alterations in the three penicillin-binding-proteins PBP1a, PBP2b and PBP2x in the cell wall reducing the affinity for β-lactam antibiotics. Resistant PBP allels are obtained by *Sp* from commensals such as *S. mitis* and *S. oralis.* A new PBP-subtype classification system has been proposed, where *Sp* susceptibility can be predicted from the exact PBP amino acid sequence. For the study, we will use whole genome sequence (WGS) data from *Sp* and *S. pseudopneumoniae, S.mitis* and *S.oralis* isolates, sent to Statens Serum Institut as well as isolates available at GenBank. For *Sp* isolates, we will describe the proportion of isolates with *essential agreement* (correct MIC), *category agreement* (correct S, I and R), *major discrepancy* (sensitive isolates are predicted intermediate or resistant) and *very major discrepancy* (resistant or intermediate isolates are predicted sensitive). For *S. pseudopneumoniae* and *S. mitis group* isolates, we will compare phenotypical susceptibility with closest matching PBP subtype. Being able to predict β-lactam susceptibility in clinical specimens, where *Sp* was only detected by PCR, e.g. because of prior antibiotic treatment would be important both for the treatment of the patient and for surveillance of pneumococcal resistance.

Reproducerbarhed af urindyrkninger i et Klinisk Mikrobiologisk laboratorium

Kristina Træholt Franck[1,2], Hanne Wise Hallberg[1], Jannie Søes[1], Christian Salgård Jensen[3], Jette Nygaard Jensen[1], Dennis Schrøder Hansen[1]

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2) Virus & Mikrobiologisk Specialdiagnostik, Statens Serum Institut
3) Klinisk Mikrobiologisk Afdeling, Slagelse Hospital

Traditionelt anvendes primært eksterne referenceprøver til kvalitetssikring i klinisk mikrobiologiske dyrkningslaboratorier. Disse prøver får imidlertid ofte mere opmærksomhed end rutineprøver, hvorfor de ikke er repræsentative for laboratoriets formåen.

Vi præsenterer en metode til at monitorere reproducerbarheden af rutinemæssig urindyrkning. Metoden kan anvendes som et supplement til den eksterne kvalitetssikring.

En bioanalytiker i prøvemodtagelsen udvalgte to tilfældige urinprøver dagligt (undtagen på ferie- og fridage) fra oktober 2016 til juni 2017 (N = 105). Urinprøverne blev delt i to (en originalprøve og en kontrolprøve), som blev analyseret separat. Kontrolprøven var ikke blindet, men bioanalytikerne i urindyrkningslaboratoriet kunne ikke se hvilken originalprøve, der hørte sammen med kontrolprøven. Oplysninger om patientalder, køn, hospitalsafdeling og prøvemateriale (fx midtstråleurin eller urin fra nefrostomikatheter) blev manuelt kopieret fra originalprøven til kontrolprøven af en sekretær. Begge urinprøver blev udsået og aflæst ved hjælp af Kiestra (BD). Dyrkningsresultater blev fortolket i henhold til vores lokale instruks.

Ved resultatgennemgang kunne dyrkningsresultatet af 1 kontrolprøve ikke findes. Af de 104 prøver, rapporteredes identisk vækst af uropatogener i signifikant mængde i 90 % (N = 94) af prøverne og vækst af forskellige uropatogener i signifikant mængde i 10% (N = 10) af urinprøverne. Denne diskrepans skyldes muligvis prøvehåndtering, idet originalprøverne blev overført direkte til Kiestra'en umiddelbart efter modtagelsen i laboratoriet, mens kontrolprøverne af praktiske grunde opbevaredes ved 5°C op til 7 timer inden udsåning og inkubering begyndte. Dette vil blive undersøgt nærmere.

DANSK SELSKAB FOR KLINISK MIKROBIOLOGI

Impact of bead beating or heat incubation prior to DNA extraction on diagnostic microbiome results from bronchoalveolar lavage samples – a pilot study

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Background: Choice of DNA extraction methods have been shown to influence results from microbiome analysis pipelines in many sample types. Statens Serum Institut has implemented a diagnostic microbiome platform which includes bacterial, fungal and parasite microbiome analysis. In order to optimise the ability to detect mycobacterium we wanted to improve the current DNA extraction strategy with a step designed to lyse the mycobacterium. To investigate what impact this would have on the results in general, we performed a pilot study with bronchoalveolar lavage (BAL) samples.

Methods: A bead-beating step or heat incubation step prior to DNA extraction with the Qiagen Blood and Tissue kit were compared to the Qiagen kit alone. Microbiome sequencing was obtained using the SSI PCR and Illumina sequencing pipeline. BIONmeta was used to classify reads. R with the package phyloseq was used for data-analysis.

Results: 23 unselected diagnostic BAL samples and one negative control (extraction buffer) were included. Abundance, diversity and evenness were all influenced by choice of extraction protocol. Introducing a bead beating step prior to DNA extraction resulted in markedly more samples positive for fungi.

Conclusions: In this pilot study we found that choice of DNA extraction protocol markedly influences the results from clinical BAL samples. This has to be investigated further.