Novelties in Clinical Bacteriology: from basics to practice

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The Year in Clinical Microbiology

Amsterdam, 11 April 2016
Dysbiosis: shift in paradigm of urinary tract diseases

New tricks for bacterial control
- OMVs- vaccine
- Fosfomycin-prostatitis

News in Bacterial Infectious Potential
- Legionella
- Klebsiella pneumoniae

Dysbiosis: shift in paradigm of urinary tract diseases
- non-sterile urine @ culturomics and metagenomic approaches
Probable Person-to-Person Transmission of Legionnaires' Disease

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and Others

News in Bacterial Infectious Potential

• Legionella
• Klebsiella pneumoniae
Legionnaires’ disease is an often severe form of pneumonia caused by *Legionella* sp.

- Typically acquired by susceptible persons (e.g., elderly persons and smokers)
- Transmission through inhalation of contaminated aerosols (e.g. cooling towers; fountains; showers)
- No person-to-person transmission

Probable Person-to-Person Transmission of Legionnaires’ Disease


Epidemiological data

- 48-year-old man (Patient 1), a smoker; maintenance worker at industrial cooling tower complex in Vila Franca de Xira
- 74-year-old woman (Patient 2), mother of Patient 1, previously healthy. Leaving in Porto

Timeline of the events consistent with the typical incubation period of Legionnaires’ disease (i.e. Patient 2 developed symptoms 1 week after the close contact with Patient 1)
Laboratorial data
- Legionella antigens positive in urine specimens from both patients
- *L. pneumophila* serogroup 1 identified in culture from respiratory secretions
- ST1905 -based typing (SBT).
- No nucleotide differences ~ 3.47 Mb of the genome sequence (whole genome sequencing)

Further laboratorial and epidemiological data
- ST1905 identified in patients during the cluster period and from the cooling tower
- Patient 2 had never been to Vila Franca de Xira, and during the cluster period, no additional cases of Legionnaires’ disease occurred in Porto.
- Water samples from the bathroom and the kitchen and a swab of the shower drain from the patient’s house were negative for *legionella*. Patient 1 did not take water from Vila Franca de Xira to Porto.
Probable Person-to-Person Transmission of Legionnaires’ Disease


Main messages

- Overall data suggests Person-to person transmission - important extension of transmission routes for Legionnaires disease - consequences for prevention and control.

- Enhanced virulence features of this strain favoring Person-to-Person transmission?

Further studies would clarify the possibility of particular virulence features that could be associated with a possible enhanced potential for person-to-person transmission.
Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health

Kathryn E. Holt⁶,⁷,¹, Heiman Wertheim⁵,⁶, Ruth N. Zadoks⁵,¹, Stephen Baker⁶, Chris A. Whitehouse⁶, David Dance⁵,⁶, Adam Jenney⁵, Li Yang Hsu⁶, Juliette Severin⁵, Sylvain Brisse⁶, Hanwei Cao⁵,⁷, Jonathan Wilksch⁵,⁷, Claire Gorrie⁵,⁷, Mark B. Schultz⁵, David J. Edwards⁶, Kinh Van Nguyen⁵, Trung Vu Nguyen⁶, Trinh Tuyet Dao⁵, Martijn Mensink⁵, Vien Le Minh⁶,⁷, Nguyen Thi Khanh Nhu⁵,⁴, Constance Schultz⁵,⁷, Kuntaman Kuntaman⁵, Paul N. Newton⁵,⁷, Catrin E. Moore⁶,⁷, Richard A. Strugnell⁵,⁷, and Nicholas R. Thomson⁵,¹

Holt et al., E3574–E3581 | PNAS | Published online June 22, 2015
Raise of *Klebsiella pneumoniae* in human infections

- Frequent cause of **nosocomial infections**
- Emergent agent of **severe community-acquired infections** (pyogenic leaver abscess, pneumonia and meningitidis)

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**Trends of MDR *K. pneumoniae* invasive isolates, EU/EEA, 2006-2014**

**Klebsiella pneumoniae isolates, USA, 1999-2010**

- 3rd Gen. Cephalosporins
- Carbapenems

[www.ecdc.eu](http://www.ecdc.eu)
Whole-genome supports KpI, KpII, and KpIII as distinct species within *Klebsiella pneumoniae* complex

**Collection**
- **288 isolates** + 40 available *K. pneumoniae* genomes
- Mainly human and bovine; nonhuman primates and marine mammals

**Klebsiella pneumoniae complex**

- *K. pneumoniae* subsp. *pneumoniae* (KpI)
- *K. pneumoniae* subsp. *rhinoscleromatis* (KpI)
- *K. pneumoniae* subsp. *ozaenae* (KpI)
- *K. quasipneumoniae* subsp. *quasipneumoniae* (KpII-A)
- *K. quasipneumoniae* subsp. *similipneumoniae* (KpII-B)
- *K. variicola* (KpIII)

**Acquired AMR genes**
- SHV
- FosA, *oqxAB*
- OKP
- FosA, *oqxAB*
- LEN
- FosA, *oqxAB*

Split network analysis and maximum likelihood (ML) phylogenetic analysis of SNPs
Whole-genome supports KpI, KpII, and KpIII as distinct species within Klebsiella pneumoniae complex

KpI, KpII, and KpIII - discrete bacterial populations that are evolving independently, with limited homologous recombination between groups.

KpI (K. pneumoniae), KpII (K. quasipneumoniae), and KpIII (K. variicola) - distinct species
**Klebsiella pneumoniae complex phylogroups: habitats and association to infection**

**KpI (K. pneumoniae)**
- Widely distributed
- Frequently carried asymptotically in humans and animals
- Most frequent cause of infections

**KpII (K. quasipneumoniae)**
- Strongly associated with humans (mostly with colonization)

**KpIII (K. variicola)**
- Environmental bacterium (mainly in plants)
- Frequent in the bovine rumen
- Infections in animals and humans.

Yersiniabactin is strongly predictive of infection, particularly in the community

Holt et al., E3574–E3581 | PNAS | Published online June 22, 2015
Most virulence genes were detected only in KpI, except for kfuABC and allantoinase.

**Predictors of Invasive infection**

Frequency of virulence gene clusters among KpI (K. pneumoniae) isolated from different human sources.
Iron-scavenging systems is central to the ability of *K. pneumoniae* to cause invasive disease in nonimmunocompromised patients.

Hospital infections mainly caused by isolates with high AMR; increasing occurrence of yersiniabactin.
The ability to cause invasive CA infections is not determined by lineage per se but may be associated with a specific virulence gene profile acquired horizontally.

But, still, some common lineages are mostly associated with MDR or hypervirulence (e.g. ST258 and ST23)

Invasive isolates were differentiated from noninvasive isolates of the same lineage by the presence of rmpA and siderophores.

- Most (74%) of the 157 KpI phylogenetic lineages were observed only once
- Variability within clones defined by MLST

KpI (Klebsiella pneumoniae) lineages

Holt et al., E3574–E3581 | PNAS | Published online June 22, 2015
Main messages

- First study linking the presence of specific virulence genes profiles with disease outcome.

- Convergence of virulence and AMR could further enhance the importance of this pathogen (severe and untreatable infections).

- Urgent need to enlarge population studies to other geographic regions and non-clinical niches.
New tricks for bacterial control

Vaccination with *Klebsiella pneumoniae*-derived extracellular vesicles protects against bacteria-induced lethality via both humoral and cellular immunity

Won-Hee Lee¹, Hyun-Il Choi¹, Sung-Wook Hong¹, Kwang-sun Kim², Yong Song Gho¹ and Seong Gyu Jeon³

*Experimental & Molecular Medicine (2015) 47, e183; doi:10.1038/emm.2015.59*

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www.nature.com/emm

A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria

Sandro Roier¹, Franz G. Zingl¹, Fatih Cakar¹, Sanel Durakovic¹, Paul Kohl¹, Thomas O. Eichmann¹, Lisa Klug², Bernhard Gadermaier¹, Katharina Weinzerl¹, Ruth Prass³, Achim Lass¹, Günther Daum², Joachim Reidl¹, Mario F. Feldman⁴ & Stefan Schild¹

New tricks for bacterial control

- **OMVs- vaccine**
- **Fosfomycin-prostatitis**
- **Plasmidic R to Fosfomycin**

News in Bacterial Infectious Potential

- **Legionella**
- **Klebsiella pneumoniae**
New tricks in Bacterial Control

Outer Membrane Vesicles (OMVs) = Extracellular vesicles from Gram negative bacteria

- Extracellular vesicles are a secretion and delivery system allowing the dissemination of bacterial products and interaction with the environment
  - Released also by Gram positive and Archaea
  - *syn.:* membrane vesicles, outer membrane fragments’ or ‘blebs’
New tricks in Bacterial Control

**OMV’s composition**

1) LPS, periplasmic proteins, outer membrane proteins and phospholipids

2) Cytoplasm and plasma membrane components – inner membrane proteins, DNA, RNA, ions, metabolites and signalling molecules

**OMVs attributed roles in bacterial physiology and pathogenesis**

- Horizontal gene transfer (beta-lactamases genes) - 4th HGT process
- Biofilm formation
- Intra- and interspecies communication
- Stress response
- Delivery of toxins and other biomolecules
- Killing of competing microbial cells
- Resistance to antibiotics (beta-lactamases)
- Complement absorption and immunomodulation
New tricks in Bacterial Control

K. pneumoniae infection prevention is needed

- Fatal infections caused by hypervirulent or MDR K. pneumoniae are of increasing concern

OMVs

- LPS - most abundant immune stimulating component
- Other immune-stimulating MAMPs (e.g. outer membrane porins, flagellins and peptidoglycans (Renelli et al., 2004; Bauman & Ku-ehn, 2006)

OMVs promising vaccine candidates against bacterial infections caused by: Haemophilus influenzae, Pasteurella multocida, Vibrio cholerae, enterotoxigenic Escherichia coli, Bordetella pertussis and Salmonella Typhimurium

N. Meningitis B OMVs are included in meningite B vaccine (Bexsero)
• *K. pneumoniae* (ATCC4208; pyogenic liver abscess) OMVs induced a humoral and cellular response in mice

• Mice were injected intraperitoneally with different doses of the OMV of *K. pneumoniae* ATCC 4208 every week for 3 weeks

• Lethal dose (sepsis) of *K. pneumoniae* (1.0 × 10^8 CFU) (ATCC4208) administered 7 days after the final immunization

80% of the mice survived after vaccination with 0.5μg of the OMVs and 100% of mice survived after vaccination with 1 μg of the OMVs.
New tricks in Bacterial Control

OMVs and vaccines

Main messages

- Vaccination with *K. pneumoniae*-derived OMVs effectively protected bacteria-induced lethality in a murine sepsis model.

- Characterization of the OMVs (size and composition) was lacking, namely to infer possibility of protection over other *K. pneumoniae* strains and some safety aspects.
OMV biogenesis

- Different models suggested (e.g. *Pseudomonas* Quinolone Signal model), but a conserved general mechanism amenable to regulation is unknown.
New tricks in Bacterial Control

Novel and potentially highly conserved OMV biogenesis mechanism in Gram-negative bacteria

OMV formation

OMV’s composition

1) LPS, periplasmic proteins, outer membrane proteins and phospholipids

2) Cytoplasm* and plasma membrane components – their presence is not convincingly explained by the currently accepted vesiculation mechanism

*VacJ/Yrb ABC transport system prevent PL accumulation in the outer leaflet of the OM
New tricks in Bacterial Control

OMVs + O-IMV (Outer-Inner Membrane Vesicles) with double-bilayer membrane vesicles

Incorporate both plasma membrane (PM) and cytoplasm content (FIG)

- mean diameter (40-260 nm)
- rate of production (0.2-1.2%)

* Cytoplasm Components
  - Proteins
  - DNA – resistance, virulence
  - ATP – signaling, communication
  - Ribosomal structures?

N. gonorrhoeae, P. aeruginosa, A. baumannii
New tricks in Bacterial Control

Main messages

- Production of these new O-IMVs implies the existence of another vesiculation model

- ATP description - possibility that some of the vesicle proteins requiring ATP cofactor maintain their functionality once outside the cell.

- Amount of O-IMV likely could impact on composition and activity of extracellular vesicles extract. Safety concerns?

OMVs and vaccines
Oral fosfomycin, an old molecule, an option for male UTI?

**Fosfomycin for treatment of prostatitis: new tricks for old dogs**  
Grayson et al., CID. 2015

**Oral Fosfomycin and Prostatitis**  
Davido B. and Dinh A., CID 2015

**Fosfomycin-tromethamine long-term oral therapy for difficult-to-treat chronic bacterial prostatitis**  
Ibai Los-Arcos et al., AAC posted Online 14 December 2015

New tricks for bacterial control

- OMVs- vaccine
- Fosfomycin-prostatitis

News in Bacterial Infectious Potential

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New tricks in Bacterial Control

**Fosfomycin**

**Few antibiotics available for prostatitis treatment**
- Poor penetration of many antimicrobials (fluorquinolones; SXT antibiotics of choice)
- Increasing incidence of multidrug resistant Gram-negative bacilli (MDR-GNB)
- Fosfomycin has been reported (parenteral agents frequently co-administered)

![Phosphonic acid derivative](https://example.com/phosphonic-acid-derivative.png)

- **Inhibits cell wall synthesis**
- **Bactericidal Activity:** Gram - and Gram +
- Recommended for uncomplicated UTI;
- Intravenous use (e.g. MDR *K. pneumoniae*)
- Levels in prostatic tissue: 4μg/ml after 3g in uninflamed peripheral prostate region
2 Patients with prostatitis. Relapse after carbapenem or parenteral fosfomycin. Switch to oral fosfomycin.

MDR E. coli. Fosfomycin MIC 1.0 mg/L (E-test)

Clinically and microbiologically cured with oral fosfomycin 3 g once-daily during 12-16 weeks

Davido & Dinh., CID 2015

12-16 weeks of oral fosfomycin questionable in a stewardship era

Retrospective study. 15 patients with chronic bacterial prostatitis with failure of prolonged antibiotic therapy. Switch to oral fosfomycin 3 g/72h for 6 weeks

MDR E. coli or K. oxytoca. Fosfomycin MICs not determined.

47% showed clinical cure.

Reasons for failures:
- MICs higher than 4μg/mL?
- Decreased fosfomycin activity in alkaline pH (pH8.5)
- Prostatic calcifications were present in 57%- biofilm formation difficult to eradicate.

Ibai et al., AAC (online 14 December 2015)

Grayson et al., CID. 2015

ESCMID eLibrary by author
Main messages

- Oral fosfomycin could be an option for male urinary tract infection in patients with MDR infection and resistance or side effects to first-line drugs.

- Further studies are needed to establish the optimal dose and duration of fosfomycin in prostatitis.
Old molecule, new tricks for bacterial control?

Fosfomycin Resistance

- Fosfomycin resistance rates: <10%
- Higher (15-34%) in carbapenemase-producing isolates

Plasmid-mediated fosfomycin resistance

- Mutations in transporter genes *glpT*, *ulpT*
- Mutations in target gene (*murA*)

Very low surveillance
Not routinely tested in many laboratories

Epidemic IncFII, IncI1 plasmids; highly diverse *E. coli* populations

*fosA*, *fosA3*, *fosB*, *fosC*
Old molecule, new tricks for bacterial control?

Importation of Fosfomycin Resistance fosA3 Gene to Europe

Ana C. Mendes, Carla Rodrigues, João Pires, José Amorim, Maria Helena Ramos, Ângela Novais, Luísa Peixe

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 22, No. 2, February 2016

• 2012
• 1 *E. coli* fosA3+CTX-M-15+rmtB+aac-6’-Ib-cr
• F2:A::B- plasmid; ST393 *E. coli*

• 61-year-old man
• Chronic prostatitis
• UTI infection after travel to Asia

Acquisition by international travel

Fosfomycin Resistance – First clinical cases of *fosA3*

Fosfomycin Resistance in *Escherichia coli*, Pennsylvania, USA

Hind Alrowais, Christi L. McElheny, Caressa N. Spychala, Sangeeta Sastry, Qinglan Guo, Adeel A. Butt, Yohei Doi

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 21, No. 11, November 2015

• 2010
• 1 *E. coli* fosA3+CTX-M-65+rmtB
• F2:A::B- plasmid

• Woman with multiple hospitalizations (2007-2010)
• Peritoneal dialysis
• UTI without travel history to Asia - No fosfomycin

Importation by food products?

ESCMID eLibrary by author
Dysbiosis: shift in paradigm of urinary tract diseases

- Legionella
- Klebsiella pneumoniae

New tricks for bacterial control

- Fosfomycin-prostatitis
- OMVs- vaccine

The new world of the urinary microbiota in women
Linda Brubaker, MD, MS; Alan J. Wolfe, American Journal of Obstetrics & Gynecology. November 2015

The microbiome of the urinary tract—a role beyond infection

Outline

- no-sterile urine @ culturomics and metagenomic approaches
Dysbiosis: shift in paradigm of urinary tract diseases

Key aspects

1. Existence of microorganisms inhabiting urinary tract in healthy individuals
   • Non-sterile urine

2. Microorganisms inhabiting urinary tract might have a role in the development of disease

3. Effects of other body microorganisms on urologic health
1. **Existence of microorganisms inhabiting urinary tract in healthy individuals**

   - Recent identification of a microbiome in the bladder

   **Key factor: 16S rRNA gene sequencing**

Most adult women have a *resident urinary microbiome*, regardless of lower urinary tract symptoms

- Highly heterogeneous mix of bacterial genera in females
- Mainly species that never cause UTI but can include potential UTI causes
- Few studies on different groups (age; gender....)
- Significant differences between urinary microbiota of men and women
Dysbiosis: shift in paradigm of urinary tract diseases

“Normal” Microbiota

1. Existence of microorganisms inhabiting urinary tract in healthy individuals

<table>
<thead>
<tr>
<th>Healthy Women</th>
<th>Healthy Men</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacillus</strong>, <em>Prevotella</em>, <em>Gardnerella</em>, <em>Peptoniphilus</em>, <em>Dialister</em>, <em>Finegoldia</em>, <em>Anaerococcus</em>, <em>Allisonella</em>, <em>Streptococcus</em>, <em>Staphylococcus</em>, <em>Actinobaculum</em>, <em>Aerococcus</em>, <em>Corynebacterium</em>, <em>Actinomyces</em></td>
<td><strong>Corynebacterium</strong>, <em>Streptococcus</em>, <em>Veillonella</em>, <em>Prevotella</em>, <em>Anaerococcus</em>, <em>Propionibacterium</em>, <em>Atopobium</em>, <em>Staphylococcus</em>, <em>Ureaplasma</em>, <em>Mycoplasma</em>, <em>Aerococcus</em>, <em>Staphylococcus</em>, <em>Gemella</em>, <em>Enterococcus</em>, <em>Finegoldia</em>, <em>Neisseria</em>, <em>Propionibacterium</em>, <em>Ralstonia</em></td>
</tr>
<tr>
<td><em>Sneathia</em>, <em>Mycoplasma</em>, <em>Ureaplasma</em> (adolescent)</td>
<td><em>Lactobacillus</em>, <em>Streptococcus</em>, <em>Sneathia</em>, <em>Mycoplasma</em>, <em>Ureaplasma</em> (adolescent)</td>
</tr>
<tr>
<td><em>Jonquettella</em>, <em>Parvimonas</em>, <em>Proteiniphilum</em>, <em>Saccharofermentans</em> (age &gt;70)</td>
<td></td>
</tr>
<tr>
<td>Urine obtained via both transurethral catheter and suprapubic aspiration yielded similar results—discarded vulvo-vaginal contamination during sample collection</td>
<td></td>
</tr>
</tbody>
</table>

Dysbiosis: shift in paradigm of urinary tract diseases

1. Existence of microorganisms inhabiting urinary tract in healthy individuals

- **Enhanced quantitative urine culture protocol** (EQUC- 0.1 ml urine, aerobic; microaerophilic and anaerobic conditions)*

- False-negative rate of the standard clinical approach: 90%

Confirmed cell viability for majority of microbiota identified by 16S rRNA sequencing

*Hilt et al., J Clin Microbiol 2014;52:871-6
Dysbiosis: shift in paradigm of urinary tract diseases

2. Urinary Microorganisms and development disease

- Alterations in the urinary microbiota linked to urologic disease, such as **neurogenic bladder dysfunction** (NBD), **interstitial cystitis** (IC) and **urgency urinary incontinence** (UUI)

- Production of neurotransmitters – peripheral nervous system. Loss of these functions might be the cause of diseases such as overactive bladder

<table>
<thead>
<tr>
<th>Rank</th>
<th>Urotype (%) in UUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactobacillus (45%)</td>
</tr>
<tr>
<td>2</td>
<td>Gardnerella (17%)</td>
</tr>
<tr>
<td>3</td>
<td>Diverse (without a dominant genus) (13%)</td>
</tr>
<tr>
<td>4</td>
<td>Enterobacteriaceae (9%)</td>
</tr>
<tr>
<td>5</td>
<td>Gardnerella/Prevotella (9%)</td>
</tr>
<tr>
<td>6</td>
<td>Staphylococcus (3%)</td>
</tr>
<tr>
<td>7</td>
<td>Aerococcus (2%)</td>
</tr>
<tr>
<td>8</td>
<td>Bifidobacterium (2%)</td>
</tr>
</tbody>
</table>

**UUI urines tends to be more diverse than women without UUI**

**Species strongly associated with UUI**
- Aerococcus urinae
- Gardnerella vaginalis
- Lactobacillus gasseri

*Lactobacillus crispatus* was associated with controls
3. Effects of other body microorganisms on urologic health

Gut

• Lack of Oxalobacter formigenes related with the development of renal calcium oxalate stones

  O. formigenes utilizes oxalate as its primary substrate - essential for the degradation of dietary oxalate in the human body

• Increased urinary oxalate concentration in individuals lacking this bacterium

• O. formigenes administration significantly decreased oxaluria in patients with enteric hyperoxaluria or after administration of oral oxalate; unsatisfactory results in mildly hyperoxaluric patients - poor colonization?

• Other combinations of oxalate-degrading bacteria seems to have a positive impact on the reduction on urinary oxalate concentration.
Dysbiosis: shift in paradigm of urinary tract diseases

Main messages

Reevaluation of Prevention, Cause, Diagnostic, and Treatment of Urinary Diseases

Ongoing microbiota research essential for elucidation:
- Normal urinary microbiome (e.g., by genera, age, hormonal status, race, and ethnicity);
- Bacteria promoting uropathogens development (e.g. UPEC) or independently causing symptoms and/or disease

Diagnostic developments: DNA sequencing is more sensitive than a standard urine culture, but not applicable for routine urinary testing. Promising developments on culturomics (EQUC, ....)

Bacteria in the urine

- Normal Urinary microbiota
  - Health contribution

- Contributes to uropathogens development
  - Diseases contribution

- Causes symptoms/disease (e.g. UPEC)
  - Diseases contribution

Disbyosis instead of Sterile/Infection

Role for Virus and fungi?
Acknowledgments

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Thank You

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