Resistance and virulence prediction: potential and challenges

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the Institute of Microbiology
of the University of Lausanne
&
Head of Diagnostic Microbiology
at the University Hospital Center,
Lausanne, Switzerland

11 sept 2011
Conflict of interests

- Research agreement with **BD** (USA) → Phoenix / BD-Kiestra
- Research agreement with **Resistell** (Switzerland) & part of the Scientific advisory board of **Resistell** since April 2018
- Research agreement with **SUEZ-ONDEO** (France)
- Co-funder of **JeuPRO** (Switzerland), that distributes the game Krobs
# The dirty genome paradigm

## VIRULENCE
1. Definition of virulence
2. What is a virulence factor?
3. The virulence may be due to lack of control
4. The virulome
5. Important for patients?
6. How to report?

## RESISTANCE
1. Antibiotic susceptibility testing
2. Resistome
3. Added value of phenotypic assays
4. Added value of «resistome» analysis
5. Important for patients?
6. How to report?
The « dirty genome » paradigm

**Increased number of genomes**

- **Sanger sequencing**
  - First bacteria: *H. influenzae*  
  - First multicellular organism: *C. elegans*  
  - Human genome draft sequence

- **High throughput sequencing**
  - Pyrosequencing (454)
  - The Global Ocean Sampling for metagenomics
  - First personal genome of James Watson
  - Rapid sequencing during an outbreak of EHEC O104:H4

- Complete genomes  
  - Draft genomes  
  - Increased number of genomes = dirty genomes
A genome with « gaps »

Without « gap closure »

Provides rapidly a lots of information → useful in medicine

Reduced costs and efforts:
- 20% of time and effort to get a first assembly
- 80% of time and effort to have complete all gaps

> 95% of the information is available since gaps are generally due to repeated sequences (transposases, rhs, ribosomal operons, …)
The « dirty genome » paradigm

High Throughput Sequencing and Proteomics to Identify Immunogenic Proteins of a New Pathogen: The Dirty Genome Approach

Coverage ~ 94%

> 90 % of proteins could be identified by MS

Proof of principle with *Parachlamydia*:
Outbreak of fever in a print shop (humidifier)

*Greub et al. PLOS One 2009; 4:8423*
The « dirty genome » paradigm

- *Parachlamydia acanthamoebae*  
Pneumonia outbreak 1990s  
454 & Solexa sequencing – 94% covered  
  Greub et al Plos One 2009

- *Vibrio cholerae* O1 str. 2010EL-1786  
Haïtian cholera outbreak 2010  
454 & Solexa sequencing – 96% covered  
  Plos One 2010

- *Escherichia coli* O104:H4 str. LB226692  
German HUS outbreak 2011  
Ion Torrent PGM sequencing – 97% covered  
  Mellmann et al Plos One 2011
The EHEC outbreak

Enterohemorrhagic (EHEC)

E. coli O104:H4

May 2011:
- 3801 cases in Germany*
  - 834 hemolytic-uremic syndrome
  - 46 deaths

* up to 21st June 2011
Enterohemorrhagic (EHEC) 

E. coli O104:H4

Sequencing done

Start sequencing

First assembly released

PCR available

Useful to develop a strain specific PCR

Mellmann et al PLOS One 2011
The EHEC outbreak

Enterohemorrhagic (EHEC)

*E. coli* O104:H4

Identified virulence factors:

- Shiga toxine = verotoxine
- Two new plasmids:
  - Coding for *AAF/I* fimbriae (83 kb) = adhesin
  - Coding for *TEM-1* & *CTX-M-15* beta-lactamases (90 kb) = resistance to antibiotics

Useful to identify virulence factors

Mellmann et al PLOS One 2011
The EHEC outbreak

**Events**

- Retrospectively, epidemics starts
- Increase in HUS and BD
- Shiga-toxin producing *E. coli* diagnosed & Pure culture available
- Sequencing starts HUSEC panel PCR
- PGM sequencing completed
- Illumina sequencing completed
- Optical mapping completed
- 454 mate-pair sequencing completed & HUSEC amplified by PCR on sprouts
- Development of a specific PCR based on genome comparisons
- Genomic characterization of strain LB226692 based on PGM sequencing
- Genomic characterization of strain C227-11 based on PacBio sequencing
- Genomic characterization of strain TY2482 based on PGM and 454 sequencing

**Applications – Proof of principle**

- First draft genome released by NCBI: Full genomics in less than 36 hours.
- Genomics to develop diagnostic tools. Qin et al.
- Rapid genomic characterization during an outbreak. Information on virulence and resistance encoding genes. Mellmann et al.
- Rapid genomic characterization during an outbreak. Information on virulence and resistance encoding genes. Razo et al.
- Open source genomics. Genomic characterization and information on virulence and resistance encoding genes. Rhode et al.
Genomic of medical importance
Institutional project since February 2012

For selected cases:
- Pathogenicity
- Clinical picture
- Outbreak
- Specific AB resistance
- Need of diagnostic tools

Goal: results in 48 to 72 hours

Clients:
- microbiologists
- medical doctors

Fragment analyzer
MiSeq (Illumina)
Institutionnal project

Day 0
- Culture
- Blood
- Sputum
- Urine
- Faeces
- Synovial fluid

Day 1
- Antigen detection
- POCT-PCR
- Gram stain
- Culture

Day 2
- Classical diagnostic PCR
- MALDI-TOF
- Antibiotic susceptibility on broth/agar

Day 3-7
- PCR & seq of resistance
- Biochemical pattern (Phoenix, Vitek)
- MLST
- RFLP
- PFGE
- Typing & phylogeny
- Resistance database
- Virulence database

Serology
- Library prep & sequencing
- Assembly & annotation
- Detailed comparison & analyses

RESISTOME & VIRULOME

✿ Directly from samples
★ From isolated colonies

Bertelli et Greub 2013 CMI
**The dirty genome paradigm**

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S. aureus

COLONISER

PATHOGEN
**S. aureus**

Adhesion to host cells
- Coagulase
- Protein A
- Leucocidin
- Proteases
- ...

**Host defenses**
- Skin barrier
- Neutrophils
- Monocytes
- Macrophages
- Immunity

**VIRULENCE FACTORS**

© ESCMID eLibrary by author
Virulence is an attribute
- of strain within a species (PVL + strains)
- of a species within a bacterial genus (S.aureus)

Several virulence factors may be combined
Sometimes, virulence only corresponds to a toxin

C. tetanii neurotoxin
S. aureus enterotoxin
Virulence
- Structure (capsule, ...)
- Toxins
- Adhesins
- Enzymes
- Superantigens
- ...

Pyogenic versus toxigenic infections

https://www.vfdb.chlamdb.ch/
The dirty genome paradigm

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Pyogenic bacterial infections and toxins

*Escherichia coli*

**fimbriae**

One bacterial species
→ multiples types of infection

Cystitis, pyelonephritis, gastointestinal infections, wound infections, ...

Uropathogens: presence of fimbriae (adhesion to urothelium)
Escherichia coli

Commensal of lower gut

Some pathogenic strains
- EIEC = invasive
- ETEC = toxigenic
- EPEC = pathogenic
- EHEC = hemorrhagic
- Urinary tract infection (90% of cystitis in women)
- Meningitis in newborns

Standard tool for genetic manipulation
(strain K12 + derivatives)
**E. coli** K12: horizontal transfer

**Mobile / repeated elements:**

- Many insertion sequences (IS)
- 3 cryptic phages
- Phages « remnants »
- Additional copies of previously reported repeated elements
- Rhs elements (recombination hot spots)
- Regions of atypical base composition

Foreign DNA

**Fluid genome dynamic**

= mechanism to acquire pathogenicity determinants

Explain the diversity of pathovars (EIEC/EPEC/ETEC/…)
**E. coli : pathogenicity**

EIEC = enteroinvasive

Similar to *Shigella* spp.

*Shigella* genus (1940) is indistinguishable from *E coli* but smaller infectious dose needed with *S. dysenteriae, S. flexnerii, S. sonnei*, and *S. boydii*

EIEC and Shigella possess a 220 kb plasmid pINV that encodes a **type III secretion system** (*mxi/spa* genes) (different from the LEE type III secretion system)

*⇒ T3SS secretes Ipa = invasin that confers the invasive phenotype (towards epithelial cells)*
**Shigella**

Non motile, Shiga-enterotoxin

Do not use lactose / maltose / xylose as carbon source

**Dysruction of cadA gene:** conversion lysine to cadaverine
(cadaverine reduce effect of Shiga enterotoxin)

**Loss of ompT:** degrade plINV-encoded VirG
(spread to adjacent epithelial cells)

Many pseudogenes (372 in strain 2457T)
due to:
- IS elements interruptions
- premature stop codons
- truncations, frameshifts

Genome decay might explain the increased pathogenicity
and the strict human specificity of *Shigella*
Conclusion

Pathogenicity is multifactorial and may arise from:
- Presence of virulence factors or from
- Gene decay
The dirty genome paradigm

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The virulome represents all the virulence factors

(including some virulence-encoding genes that are part of the core genome)

~ 2000 proteins, including the catalase encoding gene
~ 80% of S. aureus genome

4 strains carrying tsst-1
- N315
- ED133
- Mu3
- Mu50

Core genome

Variable genome
**Red:** conserved in less than 95% of the 55 genomes

**BUT** we are mainly interested in virulence factors that are the attribute of a strain (variable genome)
What are the important virulence factors?

https://www.vfdb.chlamdb.ch/
Bacteriophages in bacterial genomes

Phages (even when defective) may encode genes that represent virulence factors.

<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th>Virulence factor</th>
<th>Bacterial host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Converting beta 7888</td>
<td>Diphtheria toxin</td>
<td>Coryn. diphteriae</td>
</tr>
<tr>
<td>phiP27</td>
<td>Shiga toxin 1</td>
<td>Shigella</td>
</tr>
<tr>
<td>O-beta-1</td>
<td>Shiga-toxin 2e</td>
<td>E. coli</td>
</tr>
<tr>
<td>saPA1</td>
<td>Heat-labile toxin</td>
<td>E. coli</td>
</tr>
<tr>
<td>PS42D</td>
<td>Toxic shock syndr.</td>
<td>S aureus</td>
</tr>
<tr>
<td>PhiETa</td>
<td>enterotoxin</td>
<td>S aureus</td>
</tr>
<tr>
<td>PhiPVL</td>
<td>exfoliatine A</td>
<td>S aureus</td>
</tr>
<tr>
<td></td>
<td>Leucocidin</td>
<td>S aureus</td>
</tr>
<tr>
<td>...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Important in virulence

Casjens et al (in Bacterial chromosome, chapter 3)
How to identify virulence factors?
(apart from homology search in different databases)

Phages (even when defective) may encode genes that represent virulence factors.

Important in virulence: Casjens et al. (in Bacterial chromosome, chapter 3)

How to identify virulence factors?
(apart from homology search in different databases)
S. pyogenes cumulative nucleotide skews shows the presence of phages (grey) and virulence genes (in blue)

Panchaud et al.
BMC Genomics 2009, 10:198
S. pyogenes

TA skew on 12 different genomes

presence of phages (grey) + non-phagic steep slope (orange)

Panchaud et al.
BMC Genomics 2009, 10:198
Bacteriophages in bacterial genomes

Conclusion:

Major importance of phages in:
- Bacterial virulence
- Compaction of bacterial genomes
- Recombination events
- Preventing loss of function through mutations …

May be detected using TA skew analysis
The dirty genome paradigm

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Corynebacterium diphtheriae

*C. diphteriae* infection in a refugee documented by culture (MALDI-TOF)

- Negative result by PCR targeting the toxin encoding gene
- Severe cytopathogenicity seen at bronchoscopy (prophage virulence factor)
- Responded to treatment with erythromycin

Does this bacterium contain the prophage and its classical toxin?
Abscesses due to *S. aureus* strains, as observed in a young Eritrean male

Panton-Valentine leucocidin (PVL)?

Important?
- Mainly to understand the pathogenesis
- If not due to a PVL + strain, then we may look for immune deficit
- Epidemiology

Jaton et al 2018
Results: Whole genome phylogeny (~75% of the genome)
# The dirty genome paradigm

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Superantigens

Bind to Vβ chains of the TCR
Activation of many T cells
(up to 20% of T cells)
Massive production of cytokines
(IL-1, IL-2, TNF-α, IFN-γ)

Rash / shock

Bisno Al, Lancet Infect Dis 2003
No TSST-1 toxin detected

Decision to do the full genome

No TSST-1, no exfoliative toxin but several superantigen-like proteins
Current CHUV report

Analysis Number: 11
Sample Received: 08.25.2019
Time of Report: 08.25.2019
Lab Contact: Gilbert Grosbli 079 555 17 96
Claire Bertelli 079 555 29 50
Gilbert.Grosbl@chuv.ch
Claire.Bertelli@chuv.ch

SAMPLE DATA
MOLS number: 1908230948
Patient name: LE VECQ
Birth date: 06.30.1947
IPP: 61525
Sample type: Isolate
Sample source: Blood culture from stabus puncture

VIRULENCE
No significant hits

ANALYSIS DETAILS
Analysis number: 11
Method: Whole Genome Sequencing
Whole Genome Sequencing: n/a
Sequencing: NGS, paired end, 250bp
Bioinformatics pipeline: www.github.com/reagenten/hcag_pipelines/#1.1.0
Traceability: Parameter file: 20190219_traphylococcietermine_virulence/config.yaml

COMMENTS
-

RESULTS SUMMARY

The genes encoding for exotoxins (eta, etb), the toxic shock syndrome toxin (tst) and the Panton-Valentine laesococcidin (lvs, lvp) were not identified in the genome of the isolate.

INDICATION

Research of S. aureus strains by whole genome sequencing in context of a septic shock and pneumonia in a drug user. Suspicions of opsonophagocytosis.

VALIDATION

Date and signature:
Name: Claire Bertelli, PhD
Bioinformatics head
Date and signature:
Name: Gilbert Grosbli, Prof., MD, PhD
Laboratory head
**Sample Data**

<table>
<thead>
<tr>
<th>MOLIS number</th>
<th>Patient name</th>
<th>Birth date</th>
<th>IPP</th>
<th>Sample type</th>
<th>Sample source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1908250942</td>
<td>LE VERT, ALBERT</td>
<td>06.08.1987</td>
<td>213055</td>
<td>Isolate</td>
<td>Blood culture from venous puncture</td>
</tr>
</tbody>
</table>

**Indication**

Research of *S. aureus* toxins by whole genome sequencing in context of a septic shock and pneumonia in a drug user. Suspicion of spondylodiscitis.

**Results Summary**

The genes encoding for exfoliative toxins (eta, etc), the toxic shock syndrome toxin (tst) and the Panton-Valentine leucocidin (lukF-PV/lukS-PV) were not identified in the genome of the isolate.

**Analysis Details**

<table>
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<tr>
<th>Analysis number</th>
<th>Method</th>
<th>Whole Genome Sequencing</th>
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<tbody>
<tr>
<td>Sequencing</td>
<td>Illumina MiSeq, paired-end, 250bp</td>
<td>n/a</td>
</tr>
<tr>
<td>Bioinformatics pipeline</td>
<td><a href="http://www.github.com/metagenlab/diag_pipelines">www.github.com/metagenlab/diag_pipelines</a> #v2.1.0</td>
<td>Parameter file: 20190219_staphylococcus_aureus_virulence/config.yaml</td>
</tr>
<tr>
<td>The dirty genome paradigm</td>
<td></td>
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</table>
Disk diffusion test (Kirby-Bauer)

Mesure du diamètre de la zone d’inhibition
Minimal inhibitory concentration (MIC)
MIC now measured using e-test
Automated AST

It still takes time (rely on bacterial growth)
Look for resistance-encoding gene

- *mecA, mecC* (*S. aureus*)
- Mutation in the *rpoB* gene (*Mycobacterium tuberculosis*)
- KPC, NDM, VIM, OXA,… (Gram neg)

Reagents compartments (extraction, purification, amplification)

Optical detection

Real-time PCR (thin to allow temperature changes)

Valve (fluid movements)
Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.
Clin Microbiol Infect. 2017

Short time to results (no need of bacterial growth)
The dirty genome paradigm

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Core genome

Variable genome

RESISTOME

Part of core genome

Natural resistance deduced from identification

Selected upon AB exposure

Mobile genetic elements

Epidemiological risk
- Mutations
  Chromosomal DNA
  (10-20% of all resistances)

- Genes transfer
  - transformation
  - transduction
  - transposon (Gram positive bacteria)
  - conjugation (plasmids)
  - cassettes
  - genomic islands

With «dirty genome», no clue on the location of a given resistance-encoding gene
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</table>
Carbapenemase detection

Loss of peaks at 476 Da, 498 Da and 521 Da

Burckhardt et al., J Clin Microbiol (2011) 49: 3321-4
Carbapenemase detection: better than PCR

Modified test

using a 10 µg ertapenem disk; only 1 hour incubation

<table>
<thead>
<tr>
<th>Diagnostic approach</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic detection (Modified Hodge test)</td>
<td>90.5% (19/21)</td>
<td>86% (24/28)</td>
</tr>
<tr>
<td>Phenotypic detection (IP/IPI)*</td>
<td>54.5% (6/11)</td>
<td>92.1% (35/38)</td>
</tr>
<tr>
<td>PCR-Check MDR Carba</td>
<td>90.5% (19/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>Microarray</td>
<td>90.5% (19/21)</td>
<td>100% (28/28)</td>
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<tr>
<td>MALDI-TOF</td>
<td>100% (21/21)</td>
<td>100% (28/28)</td>
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<tr>
<td>MS/MS</td>
<td>100% (21/21)</td>
<td>100% (28/28)</td>
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*phenotypic detection of metallo-β-lactamases (VIM, IMP, NDM)
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Genome sequencing and AB susceptibility

Ellington et al., Clin Microbiol and Infection, 2017
Genome sequencing and AB susceptibility

1. Poor or non-existing evidences to use genomics to predict AB resistance
2. To assess genotype-phenotype concordance, use epidemiological cut-off (instead of clinical beak-points)
3. Harmonization of analytical pipeplines & quality metrics needed
   
   Number of reads, average read length, depth of coverage (chromosome-plasmid)
   Proportion of reads mapped to reference genome, assembly size, number of contigs, >500 bp, longer contig, N50, ….

4. Single public database of all known resistance loci is needed

Main limitations:
- Cost
- Time to results
- Dependency on culture
- Gap in current knowledge (efflux pumps, expression of OMPs, LPS, …)

→ Insufficient evidence to support use of WGS-inferred AST to guide clinical decisions

Ellington et al, Clin Microbiol and Infection, 2017
Escherichia coli and other enterobacteriaceae

> 95% concordance between genotype & phenotype
  Stoesser et al J Antimicrob Chemoth 2013
  Zankari et al J Antimicrob Chemoth 2013
  Tyson et al J Antimicrob Chemoth 2015

But:

Modification of membrane permeability
Upregulation of efflux pumps

Difficult to predict

Plasmids assemblies are difficult with short reads data

Pecora et al Mbio 2015
Ellington et al CMI 2017
*Pseudomonas aeruginosa*

91% sensitivity and 94% specificity to predict resistance to meropenem and levofloxacin

Only 60% concordance for aminoglycosides

Kos et al AAC 2015

But:

Challenges due to expression of genes encoding efflux pumps & beta-lactamases

Elington et al CMI 2017
**Acinetobacter**

High concordance to predict resistance to Carbapenems & aminoglycosides (n=75)  
Wright et al IGE 2015

100% sensitivity & 100% specificity of ARG-ANNOT (n=174)  
Gupta SK et al AAC 2014

But:

Challenge due to expression of genes encoding efflux pumps & beta-lactamases  
Elington et al CMI 2017
**Staphylococcus aureus**

99.6% specificity & 99.1% sensitivity using Mykrobe predictor (allowing classification of Staphylococcus at species level from raw reads in less than 3 minutes)

Bradley P et al Nature Communication 2015

But:

Limited knowledge in mechanisms of resistance to aminoglycosides and glycopeptides

Chen et al J Antimicrob Chemother 2015
Mc Evoy et al AAC 2013

Risk of loss of mobile elements such as ermC or SCCmec cassette when passaging the isolate …
→ do it from primoculture or from blood culture pellet …

Tagini et al, Eur J Clin Microbiol Infect Dis, 2017
**Enterococci**

Accurate detection of *vanA*, *vanB*, … in enterococci

Howden et al Mbio 2013

Moreover:

Good concordance of phenotype (AST) with *vanA* / *VanB* detection by PCR

Huh et al Ann Lab Med 2015
Holzknecht et al NMNI 2017
### Presence of resistance genes in 78 published *K. pneumoniae* genomes:

- **Green** = on plasmid
- **Red** = on chromosome

#### MAH strain

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<th>Gene</th>
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<th>blaOXA-48</th>
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**Collaboration with P. Nordmann**

Trestan et al
The dirty genome paradigm

VIRULENCE
1. Definition of virulence
2. What is a virulence factor?
3. The virulence may be due to lack of control
4. The virulome
5. Important for patients?
6. How to report?

RESISTANCE
1. Antibiotic susceptibility testing
2. Resistome
3. Added value of phenotypic assays
4. Added value of «resistome» analysis
5. Important for patients?
6. How to report?
STREAMLINING
or
AB SPECTRUM BROADENING

Major impact of phenotypic AST
Clerc et al Clin Infect Dis 2013
Osthoff et al CMI 2017

Impact of PCR-based assessment of resistance
(ie MRSA vs MSSA)
Clerc et al CMI 2014
### Impact of the Sequential Reporting

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<th>Impact of MALDI-TOF MS on empirical antibiotic therapy</th>
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<td>Introduction of empirical antibiotic therapy</td>
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<td>Introduction of focused empirical antibiotic therapy</td>
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**Impact of MALDI-TOF on empirical antibiotic therapy in 35% of bacteremia**
Etude prospective randomisée

- MALDI-TOF subgroup 1
- Conventional subgroup 1
- MALDI-TOF *E. coli*/*Klebsiella spp.*
- Conventional *E. coli*/*Klebsiella spp.*

p<0.001 (Log-rank test)

Cumulative optimal treatment

Hours since blood culture draw
The dirty genome paradigm

**VIRULENCE**
1. Definition of virulence
2. What is a virulence factor?
3. The virulence may be due to lack of control
4. The virulome
5. Important for patients?
6. How to report?

**RESISTANCE**
1. Antibiotic susceptibility testing
2. Resistome
3. Added value of phenotypic assays
4. Added value of «resistome» analysis
5. Important for patients?
6. How to report?
Exemple of CHUV report

**INDICATION**

Investigation of 3 *K. pneumoniae* isolates in the context of a possible transmission, and observed phenotypic differences in resistance profiles.

**RESULTS SUMMARY**

Isolates 1803133289 and 1803152373 present no single nucleotide polymorphism (SNP) on the core genome, supporting a possible recent transmission between patients.

Isolates 1803063412 and 1803133289, from the same patient (abscess and rectal swab) present only 2 single nucleotide polymorphisms in the core genome.

All isolates belong to sequence type 512 (ST512).

The isolates present a similar antibiotic resistance profile, with a notable difference in chloramphenicol resistance (absence of catI gene in isolate 1803152373), trimethoprim resistance (dfrA12 absent in 1803152373) and macrolide resistance (Mrx and mphA absent in 1803152373). Also, the aminoglycoside acetyltransferase gene AAC(3')-Ia is absent in 1803152373. Please note that only genes conferring resistance by their presence are reported here, whereas mutations conferring resistance are not reported yet.
**VALIDATION**

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Conclusions

Dirty genome sequencing emerged in most large medical centers:

- to guide epidemiologists
- to develop diagnostic tools
- to understand pathogenesis by identifying virulence factors
- to indirectly assess antibiotic susceptibility (for fastidious organisms)
- to determine antibiotic resistance mechanisms (epidemiological concern)

More data much faster
New high-throughput sequencing technologies

Development of new diagnostic approaches

Genotyping

Taxogenomic

Therapy

Prognosis

Resistance genes

Virulence genes

Development of new diagnostic approaches

Greub G
Clin Microbiol Infect 2013

New ELISA

New PCR
Thank you

Sébastien Aeby
Florian Tagini
Onya Opota
Valentin Scherz
Claire Bertelli
Trestan Pillonel
Sacha Laurent
and many others

24 to 27th August 2020 – INTRACELLULAR BACTERIA