S. capitis: a world-wide multidrug-resistant clone responsible for sepsis in neonatal intensive care units

Pr. Frédéric LAURENT

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Centre National de Référence des Staphylocoques
Centre Internationale de Recherche en Infectiologie – INSERM U1111, CNRS UMR5308, Université Lyon 1, ENS de Lyon - Equipe "Pathogénèse des infections à staphylocoques"
Institut des Sciences Pharmaceutiques et Biologiques de Lyon, Département de Microbiologie-Mycoogie
**Staphylococcus capitis:**
- skin flora, especially on the head
- classical contaminant in wound specimens, blood cultures, ...

.... but also a true pathogen!
2007

Croix Rousse Hospital - Lyon
Northern Biology Centre

French National Reference Centre for Staphylococci
"There is a lot of S. capitis in blood cultures from Neonatal Intensive Care Unit (NICU), no ?… I never saw that before. .... It is surprising, isn’t it ?”

"Bofff (French exclamation to show that you are not enthousiastic!), we always have some isolates …”

"Maybe we have to see what’s happened. Maybe it is an outbreak ? !!!”

"Well, never minds … bofft boff… nobody is interested by this topic … and …
2007

Croix Rousse Hospital - Lyon

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…. and be careful to the two heads of the two NICUs in Lyon if you explain them they have an outbreak !!!!!!!
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Staphylocoques
Centres National de Référence

Pr J-C. PICAUD

Dangerous for your health

Not good for your young career !

Pr O. CLARIS
Late-onset sepsis (LOS) = sepsis after 3 days of life
- Frequent (>20% of hospitalized very low birth weight prematures)
- Severe
- Major cause of morbidity and mortality (Stoll et al., 2002)

Coagulase-negative staphyloccoci (Rasigade, 2012)
- Major pathogens in LOS
- S. epidermidis = the most prevalent species
- Frequent methicillin-resistance

Vancomycin as first-line antimicrobial agent
S. capitis in prematures

- Despite this ...

**PERSISTENT STAPHYLOCOCCUS CAPITIS SEPTICMIA IN A PRETERM INFANT**

Pak C. Ng, MD, FRCPCH, Viola C. Y. Chow; Cheuk H. Lee, FRCP, Julia M. L. Ling, PhD, Huil L. Wong, MRCP, and Raphael C. Y. Chan, PhD.


**Staphylococcus capitis** bacteremia of very low birth weight premature infants at neonatal intensive care units: clinical significance and antimicrobial susceptibility.


**ORIGINAL ARTICLE**

Almond oil implicated in a *Staphylococcus capitis* outbreak in a neonatal intensive care unit

C Gros-Le Gueu1,2, S Fournier1, B Andre-Richet1, J Guillon1,2,4, C Chamoux1, E Espaze1, H Richet1, JC Rose2 and B Lepelletier1,3

1Institut de Physiopathologie et Microbiologie, CHU, Nancy, France; 2Hôpital de Nancy, Centre Hospitalier Universitaire, Nancy, France; 3Unité de recherche, INSERM U527, Nancy, France; 4Unité d'Accueil Hospitalier, CHU, Nancy, France; 5Laboratoire de Bactériologie, CHU, Nancy, France.
S. capitis: clinical descriptive epidemiology

Retrospective study of prevalence 2004-2009 in HCL’s NICU

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<sup>a</sup> Comparison of NICU infants against adult ICU patients using χ² test for independence.
### S. capitis: Clinical Descriptive Epidemiology

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**S. capitis**: clinical descriptive epidemiology

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Some isolates ... ??? .... Too many
**S. capitis : clinical descriptive epidemiology**

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Some isolates ... exclusively in NICUs
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High prevalence of LOS due to S. capitis in NICUs in Lyon
**S. capitis : clinical descriptive epidemiology**

**Retrospective study of prevalence 2004-2009**

- Comparison of LOS due to *S. capitis* vs. *S. epidermidis*
- Definition of persistent bacteremia: >=3 consecutive blood culture bottles positive for the same pathogen for a period >48h

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Higher persistence of bacteremia due à *S. capitis*
Clinical study – NICU Croix Rousse Hospital, Pr J-C Picaud

• Restrospectyive study January 2006 – December 2009
• Prematures <34 weeks of gestation with LOS due to CoNS in the NICU
• ”LOS S. capitis” Group (n=74) Vs. Group ”LOS other CoNS” (n=31)
• Comparison of clinical and biological parameters
  ✓ General clinical and biological parameters: NS
  ✓ Mortality : NS
  ✓ Morbidity: composite indicators (DBP/ECUN/LMPV/HIV):
    necrotizing enterocolitis, periventricular leukomalacia, intraventricular haemorrhage, chronic lung disease requiring oxygen therapy,

Significant higher morbidity in ”LOS S. capitis” group
56% vs 32%; p=0.028
Deaths of premature infants directly related to *S. capitis* LOS
To sum up:

- High prevalence of LOS due to *S. capitis* in NICUs in Lyon
- LOS *S. capitis* associated to persistent bacteremia
- LOS associated to an increase of morbidity (mortality?)

... for a species classically described as non-virulent
... and classically considered as a member of the normal flora

"Bofff (French exclamation to show that you are not enthusiastic!), we always had some isolates ..."
S. capitis in NICUs

- Molecular typing

PFGE for pulsed Field Gel Electrophoresis
• Molecular typing

PFGE for pulsed Field Gel Electrophoresis

S. capitis in NICUs
S. capitis in NICUs

- Molecular characterization
  PFGE for pulsed Field Gel Electrophoresis

S. capitis methi-R collected from Blood culture in NICU (pulsotype NRCS-A)
Lyon, 2004-2012

S. capitis methi-R collected from Blood culture in pediatric/adult wards
Lyon, 2004-2012
**S. capitis** in NICUs

- Molecular characterization
  - PFGE for pulsed Field Gel Electrophoresis

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Lyon, 2004-2012

an endemic-epidemic strain specific to NICU in Lyon and different to **S. capitis** isolates from adults
S. capitis in NICUs

- Molecular characterization

PFGE for pulsed Field Gel Electrophoresis

S. capitis methi-R collected from blood cultures in various French NICU Lyon, Caen, St Etienne, Nantes, Versailles, Limoges, Paris, Troyes (except line 11 and 12 S. capitis meti-R from adults)
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*an endemic* clone (named NRCS-A) specific to NICU in France and different from *S. capitis* isolates from adults
Participants: 47 level-III NICUs

- Distribution of bacteremia NRCS-A *S. capitis*
  - 43 NICUs with at least one NRCS-A isolate
  - 4 NICUs: 0 *S. capitis*
  - Proportion NRCS-A *S. capitis*:
    - 0 to 46% of positive blood cultures
    - Median = 13%
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S. capitis in NICUs

- Molecular characterization

only in France?
S. capitis: international epidemiology in NICUs

- Molecular characterization

only in France?

Selection of 3 isolates / country

PFGE SacII for 14 clinical S. capitis strains

NB: similar results with Smal

International clone specifically involved in LOS in prematures!

Non-NRCS-A clinical isolates available publicly available: 7,910 to 50,723 versus souche NRCS-A.

WGS (Whole Genome Sequencing) of 2,080,323 pb analyzed.
Evaluation of NRCS-A distribution (no prevalence data!)

- Extension of isolates panel: n>200
  - > 20 countries throughout the world
  - From 1994 to 2015
- Techniques: WGS = the same clone in all NICUs with very limited number of SNPs

NRCS-A = first endemic methicillin-R CoNS clone with a worldwide diffusion and specifically present in NICU (highly premature neonates)
Evaluation of NRCS-A distribution (no prevalence data!)

- Extension of isolates panel: n>200
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ESMID Grant - ESGS

Underway: WGS of 260 genomes coming over the world collected between 1990 et 2015
- birthdate of the clone?
- Phylogeoegraphy? search for a common source?
- way of diffusion?
- specific gene of the clone? (56 genes including…. nisR)
Endemicity of NRCS-A
Why? How?

1. Antibiotic resistance pattern of
*S. capitis* NRCS-A
Multidrug resistant

unusual because *S. capitis* is usually a full susceptible species

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<td>Fusidic acid</td>
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<td>Fluoroquinolones</td>
<td>10 (4.9)</td>
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*atypical fluoroquinolone susceptibility* for MDR staphylococci but make sense with the specific antibiotic selective pressure in NICU (no use of FQ)

Antibiotic resistance pattern suggests a **NICU-specific clone** "*born in NICUs and spreading in NICUs*"
Antibiotic resistance pattern of *S. capitis* NRCS-A

Isolates with high MIC level/heteroresistance

Persistent bacteremia more frequent with *S. capitis* than other CoNS

High selective pressure vancomycin in NICUs

Decreased susceptibility to vancomycin could be the consequence of a high adaptation to selective pressure?
In vitro vancomycin resistance selection model

- **S. capitis** NRCS-A
- **S. capitis** adult
- **S. epidermidis**
- **S. aureus**

**Vancomycin**

- **day 0**: Inoculation with various strains
- **day 1**: Broth Microdilution
  - 24h incubation
  - X = MIC (minimal inhibitory concentration)
- **day 2**: Further subcultures
- **day 15**: Stability of acquired resistance
- **day 15+9**: Cross resistance with daptomycin and linezolid

- 9 subcultures vancomycin-free agar

ESCMID eLibrary © by author
Evolution of vancomycin MIC under selective pressure

Antibiotic resistance pattern of *S. capitis* NRCS-A

*S. capitis* NRCS-A acquired vancomycin resistance under *in vitro* selective pressure significantly faster than other species.

Resistance breakpoint (MIC = 2 mg/L) *(EUCAST recommendations)*

*S. capitis* NRCS-A
*S. cap. adulte*
*S. epidermidis*
*S. aureus*
Endemicity of NRCS-A
Why? How?

2. Infection route and colonization in neonates?
Infection route and colonization

→ Infection route of *S. capitis* NRCS-A in neonatal LOS?

- **Skin/catheter?** Usually recognized as important route, BUT...
  - *S. capitis* = low producer of biofilm
  - *S. capitis* LOS in the absence of catheters!

- **Gut/translocation?**
  - immaturity of preterm intestina,
  - multi-resistant CoNS = first bacteria in the gut microbiota of hospitalized neonates

Hypothesis: gut colonization ... translocation .... LOS .... ... *S. capitis* isolates to the bloodstream

Prospective cohort study concerning colonization and LOS due to *S. capitis*

- *S. capitis* colonization is a risk factor for *S. capitis* LOS ?
- Other risk factors of *S. capitis* LOS ?
Infection route and colonization

- **Prospective single-centre cohort study in a French NICU**

- **Patients**
  - Hospitalized in the NICU of the Croix Rousse Hospital (Lyon, France)
  - June 2011 - January 2012
  - For whom at least one stool culture had been performed

- **Stool samples** routinely performed at Day 0 and 1/week
  - Colonization screening: incubation on home-made agar plates selective for *S. capitis* NRCS-A
  - Identification confirmed using MALDI-TOF

- **Clinical data collection**
  - **Primary endpoint** = occurrence of *S. capitis* LOS (clinical + biological criteria)
  - ICCA software (Philips®): exhaustive and prospective encoding of the clinical data

- **Stat:** Cox model, with time-dependent covariates
Infection route and colonization

- Population n=229
  - 83 patients/229 *S. capitis* gut colonization (36%)
  - 28 patients/229 *S. capitis* LOS (12%)

- Vancomycin increases the risk of *S. capitis* gut colonization

- Factors associated with *S. capitis* LOS (multivariate)
  - Prior vancomycin administration
    - vancomycin = disruption of gut microflora barrier and selection of vancomycin non-susceptible *S. capitis* NRCS-A?
  - Negative association between duration of *S. capitis* colonization and LOS (HR = 0.25, p=0.085)
    - Acquired protective immunity in patients with prolonged *S. capitis* colonization?
Endemicity of NRCS-A
Why? How?

3. Reservoirs and ways of dissemination within NICU settings
Analysis of vaginal samples from pregnant women

Screening for *S. capitis* colonization

- Vaginal samples
  - Routinely performed for the screening of GBS
- Identification of *S. capitis* using a specific and chromogenic agar plates

![Diagram showing analysis process]

- Enrichment in BCC
- Selection on MRSA Brillance II
- Typical aspect of *S. capitis* colonies
- MALDI-TOF Identification
Analysis of vaginal samples from pregnant women

Screening for *S. capitis* colonization

- Vaginal samples
  - Routinely performed for the screening of GBS
- Identification of *S. capitis* using a specific and chromogenic agar plates

<table>
<thead>
<tr>
<th>Enrichment in BCC</th>
<th>Selection on MRSA Brillance II</th>
<th>Typical aspect of <em>S. capitis</em> colonies</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C 24h</td>
<td>37°C 5 J</td>
<td></td>
<td>MALDI-TOF</td>
</tr>
</tbody>
</table>

- 106 samples
- No *S. capitis*

Maternofetal transmission of *S. capitis* is unlikely
Environmental screening

Reservoirs and ways of dissemination

- Neonate
- Environment
- Caregivers

Reservoirs and ways of dissemination

Environmental screening

1. Around infected patients and controls

S. capitis

MRSA Brillance
After 5 days

ESCMID eLibrary © by author
### Reservoirs and ways of dissemination

#### Environmental screening

1. Around infected patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Incubators (n=12)</th>
<th>Around patients (n=11)</th>
<th>NICU equipment (n=10)</th>
<th>Total (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>7</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>17</td>
</tr>
</tbody>
</table>

*p < 0.01*

Positive samples for *S. capitis*

- 33 samples/patient
- 3 infected patients (*S. capitis* positive blood culture) and 3 controls

*Higher *S. capitis* colonization around infected patients*

*High colonization in incubators, in cases AND controls*
**Reservoirs and ways of dissemination**

**Environmental screening**

2. Throughout the setting

**23 samples/week during 6 consecutive weeks**

<table>
<thead>
<tr>
<th>Week no.</th>
<th>Near patients</th>
<th>Medical office</th>
<th>NICU equipment</th>
<th>Relaxation areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Persistence of *S. capitis* inside the NICU, especially around patients /incubators – no single/constant reservoir
Environmental screening

3. Incubator sampling before/after decontamination

9 samples/incubator
16 incubators before/after decontamination

16/16 (100%) positive for *S. capitis*
BEFORE decontamination

10/16 (62.5%) positive for *S. capitis*
AFTER decontamination

Mattress (sewing) ++
Electronic balance +++
Wipe

Partial failure of incubator decontamination protocol on the eradication of *S. capitis*
In vitro antiseptic susceptibility test

- Review of the literature:
  - Lepainteur et al, 2013: S. capitis and S. epidermidis isolates from NICU: 41% decreased susceptibility to at least one antiseptic molecule

- MICs determination
  - BAC (benzalkonium chloride)
  - CHX (chlorhexidine)
  - Microdilution method
  - 75 S. capitis NRCS-A isolates

- Results:
  - 86% decreased susceptibility BAC/CHX

High tolerance towards antiseptic molecules in S. capitis

Clinical impact? Desinfection failure?

Perspectives: to test other molecules
**Reservoirs and ways of dissemination**

**HCW screening**

*S. capitis* screening in the nose and on hands of nurses

2 anonymous samples/nurses
Control after a 5-days wash-out period

<table>
<thead>
<tr>
<th></th>
<th>Nose</th>
<th>Hands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside NICU</td>
<td>0/22 (0%)</td>
<td>3/22 (13.6%)</td>
</tr>
<tr>
<td>After &gt;5 days « wash out » period</td>
<td>0/20 (0%)</td>
<td>0/20 (0%)</td>
</tr>
</tbody>
</table>

Passive colonization when contact with patients but no persistent carriage in HCW → Passive vectors of dissemination inside NICU
Reservoirs and ways of dissemination

Hypothesis of *S. capitis* diffusion pathways inside NICU

- *S. capitis* present in the NICU environment
- Incubators contamination
- Resistance to antiseptic molecules / desinfection protocol
- Cutaneous contamination of the HCW
- Passive transmission to the neonates

If the clone invade your NICU, try to take **drastic actions** to prevent implantation, ASAP!
Yes, coagulase negative staphylococci can be true pathogens!

- **Playground**: Specifically in NICUs
- **Selection**: Multidrug-R and decreased susceptibility to vancomycin
- **Condition of spread**: Diffusion not only in France... But also endemic worldwide!
Yes, coagulase negative staphylococci can be true pathogens!

Specifically in NICUs

Multidrug-R and decreased susceptibility to vancomycin

Diffusion not only in France... But also endemic worldwide!

If failure of vancomycin therapy >48 hours, consider an alternative therapy
If the clone invade your NICU, try to take drastic actions to prevent implantation
Optimisation of disinfection of the incubators and the NICU environment
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If the clone invade your NICU, try to take drastic actions to prevent implantation

Optimisation of disinfection of the incubators and the NICU environment

How this clone has disseminated... at such a large scale?

Why these LOS occur in neonates (virulence)?
Thanks to...

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Nicu - GHN
Olivier CLARIS

Caroline BOUVEYRON
Nadia BOUDROUN
Christine COURTIER
Christine GARDON

Angela KEARNS – HPE, UK
Olivier DENIS
Margaret EYTON – Australia

And all the colleagues who sent isolates...
Endémicité du clone NRCS-A *S. capitis*: Pourquoi? Comment?

**WGS clone NRCS-A**

- PacBio
- 454

**CR01 (FR)**

**CR03 (Be)**

**CR04 (Aus)**

**CR05 (UK)**

**S. capitis**

**S. aureus**

**S. epidermidis**

**Génome référence fermé**

**Core genome du clone NRCS-A**

**Gènes spécifiques**

**Resistome**

**Virolome**

**MGEs**

**Reads**

**WGS**

ESGMID eLibrary © by author
a Gram-positive targets

Class I (e.g. nisin)

Cell wall

Lipid II

Inhibition of peptidoglycan synthesis

Pore formation

Class II (e.g. lactococcin A)

Man-PTS
Endémicité du clone NRCS-A *S. capitis*: Pourquoi? Comment?

Séquençage haut-débit (NGS) **clone NRCS-A**

- PacBio
- 454
  - CR01 (FR)
  - CR03 (Be)
  - CR04 (Aus)
  - CR05 (UK)

Génome référence fermé

Core genome du clone NRCS-A

Gènes spécifiques
- Resistome
- Virolome
- MGEs

*S. aureus*

*S. epidermidis*

*S. capitis* non-NRCS-A
Mécanisme de résistance à la vancomycine

VISA/GISA = vancomycin intermediary *S. aureus* "souche de sensibilité diminuée aux glycopeptide"
Mécanisme de résistance à la vancomycine

VISA/GISA = vancomycin intermediary *S. aureus*
"souche de sensibilité diminuée aux glycopeptide"
Take Home message

• un **clone S. capitis NRCS-A** responsable de **sepsis tardifs**
• une **diffusion mondiale**
• des souches **multirésistantes**
  
  meti-R KTGenta-R Fosfomycine-R
  
  CMI vancomycine >1 mg/L- Teicoplanine > 2 mg/L
  +/- Rifampicine-R, Ac fusidique-R

• si la vancomycine n’est pas efficace au bout de 48h, envisager une alternative

• si le clone apparaît dans un service, il faut prendre des mesures drastiques pour **empêcher qu’il ne s’installe**!

• La **désinfection des couveuses et de l’environnement est critique** pour éviter les infections nosocomiales

Pourquoi ces sepsis (virulence) ?

Comment ce clone diffuse … aussi loin ?

Quel traitement alternatif à la vanco?
S. capitis et réanimation néonatale

Take Home message

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Porte d’entrée de ces bactériémies ?

Pour étudier cette translocation digestive

Modèle cellulaire

- Adhésion
- Internalisation
- Translocation

Modèle souris
- Gavage avec *S. capitis* NRCS-A vs *S. capitis* non-NRCS-A vs *S. epidermidis*
- Suivi colonisation digestive, implantation digestive, capacité de translocation
Séquençage haut-débit (NGS) clone NRCS-A

PacBio

454

CR01 (FR)

CR03 (Be)
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