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To cite this version:

Anne Filleron, Michel Simon, Stefaniya Hantova, Gilles Cambonie, Tu Anh Tran, et al.. Detection of coagulase-negative staphylococci in pasteurized breast milk. 32nd Meeting of the European Society for Paediatric Infectious Diseases (ESPID 2014), May 2014, Dublin, Ireland. . hal-03993134

HAL Id: hal-03993134

https://hal.science/hal-03993134v1

Submitted on 16 Feb 2023

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Detection of coagulase-negative staphylococci in pasteurized breast milk

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Keywords:
Coagulase negative staphylococci (CoNS)
Breast milk
Pasteurization
Neonate
Cultivation
PCR-TTGE

BACKGROUND

❖CoNS:

- ❖ most represented group of bacteria in raw breast milk and stools of very preterm neonates [1,2]
- ❖ leading cause of late-onset sepsis in preterms [3]

➢ Similarity between CoNS isolated from stools and neonate blood cultures suggests a causal relationship [4]

➢ Preterm infants are frequently fed with pasteurized breast milk (PBM) from milk bank

➢ Pasteurization process should completely eliminate remaining CoNS

MAIN OBJECTIVE

Search for **CoNS in pasteurized breast milk**
by cultivation and cultivation-independent methods
to investigate the possible role of PBM as a vehicle
for the **transmission** of CoNS to the neonates fed with PBM.

MATERIALS AND METHODS

❖ 603 samples of PBM from milk bank

❖ **Conventional** and **optimized culture** of PBM, and *rpoB* gene-based identification of CoNS [5]

- ❖ 1 mL of sample inoculated onto sheep blood agar (bioMérieux) and incubated at 37° C during 48 h
- ❖ samples inoculated on trypticase soy agar (TS, Difco) and Schaedler broth (bioMérieux) incubated at 37° C for 10 days, TS agar slant being daily inoculated by turning, and minimal medium (Nutrient agar, Difco) into a tube, incubated for 20 days at 37° C (500 µl of milk in each of the media).

❖ **Cultivation-independent method after DNAse digestion** of free DNA:
tuf gene based-PCR followed by temporal temperature gradient gel electrophoresis (*tuf*-PCR-TTGE) [6]

RESULTS

CULTURE

❖ Conventional cultures — All negative for CoNS

❖ Optimized culture

- ❖ CoNS isolated from 7/392 samples — **1.8% of samples**
  - ✓ Identification
    - ❖ *S. epidermidis* (n=5)
    - ❖ *S. haemolyticus* (n=1)
    - ❖ *S. warneri* (n=1)
- ❖ 10 samples: Gram-positive cocci with morphology compatible with *Staphylococcus* spp. and negative sub-culture

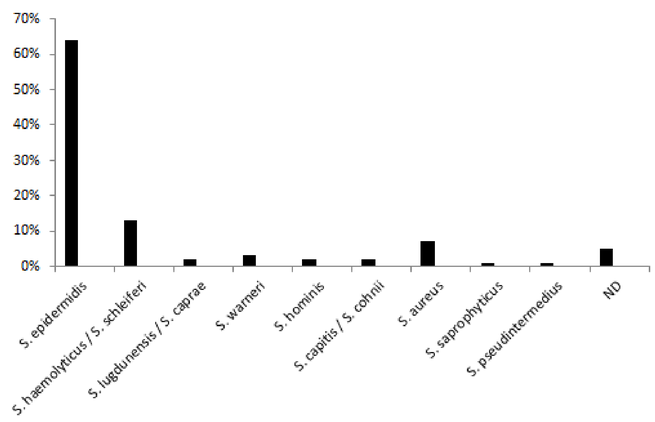
INDEPENDANT - CULTURE

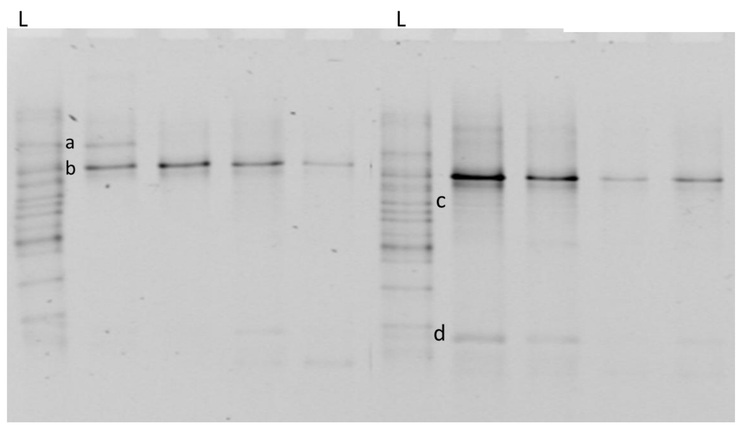
❖ *tuf*-PCR-TTGE — All positive for CoNS

❖ *tuf*-PCR-TTGE after DNAse digestion of free DNA

- ❖ Detection of CoNS DNA from 84/211 samples — **39.8% of samples**
  - ✓ Number of species by sample: 1 to 7
  - ✓ Identification
    - ❖ *S. epidermidis*: major detected species 64%
    - ❖ *S. haemolyticus* 13%.

Distribution of staphylococcal species detected in DNase-treated pasteurized milk





TTGE migration of pasteurized breast milk samples treated by DNase.
L, *Staphylococcus* diversity ladder.
Specific band for each species is noted by a letter;
a for *S. lugdunensis*, b for *S. epidermidis*, c for S. aureus, d for artifactual band.

DISCUSSION - CONCLUSIONS

❖ With culture: identification of CoNS in PBM, mainly *S. epidermidis*
AND
❖ With culture and Cultivation-independent method after DNAse digestion of free DNA:
- Detection of CoNS DNA
- Identification of one or more species of CoNS, mainly *S. epidermidis*

➢ Viable and cultivable CoNS persisted in breast milk after pasteurization.

➢ PBM could supply intestinal reservoir of CoNS species involved in preterm infant infections.

REFERENCES
[1] Jacquot, Neveu et al. 2011
[2] Jimenez, Delgado et al. 2008
[3] Stoll, Hansen et al. 2002
[4] Soeorg, Huik et al. 2013
[5] Drancourt, Raoult. 2002
[6] Filleron, Simon et al. 2014
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