



HAL
open science

Detection of coagulase-negative staphylococci in pasteurized breast milk

Anne Filleron, Michel Simon, Stefaniya Hantova, Gilles Cambonie, Tu Anh Tran, H el ene Marchandin, Estelle Jumas-Bilak

► **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-03993134>

Submitted on 16 Feb 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin ee au d ep ot et  a la diffusion de documents scientifiques de niveau recherche, publi es ou non,  emanant des  tablissements d'enseignement et de recherche fran ais ou  trangers, des laboratoires publics ou priv es.



Distributed under a Creative Commons Attribution 4.0 International License

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

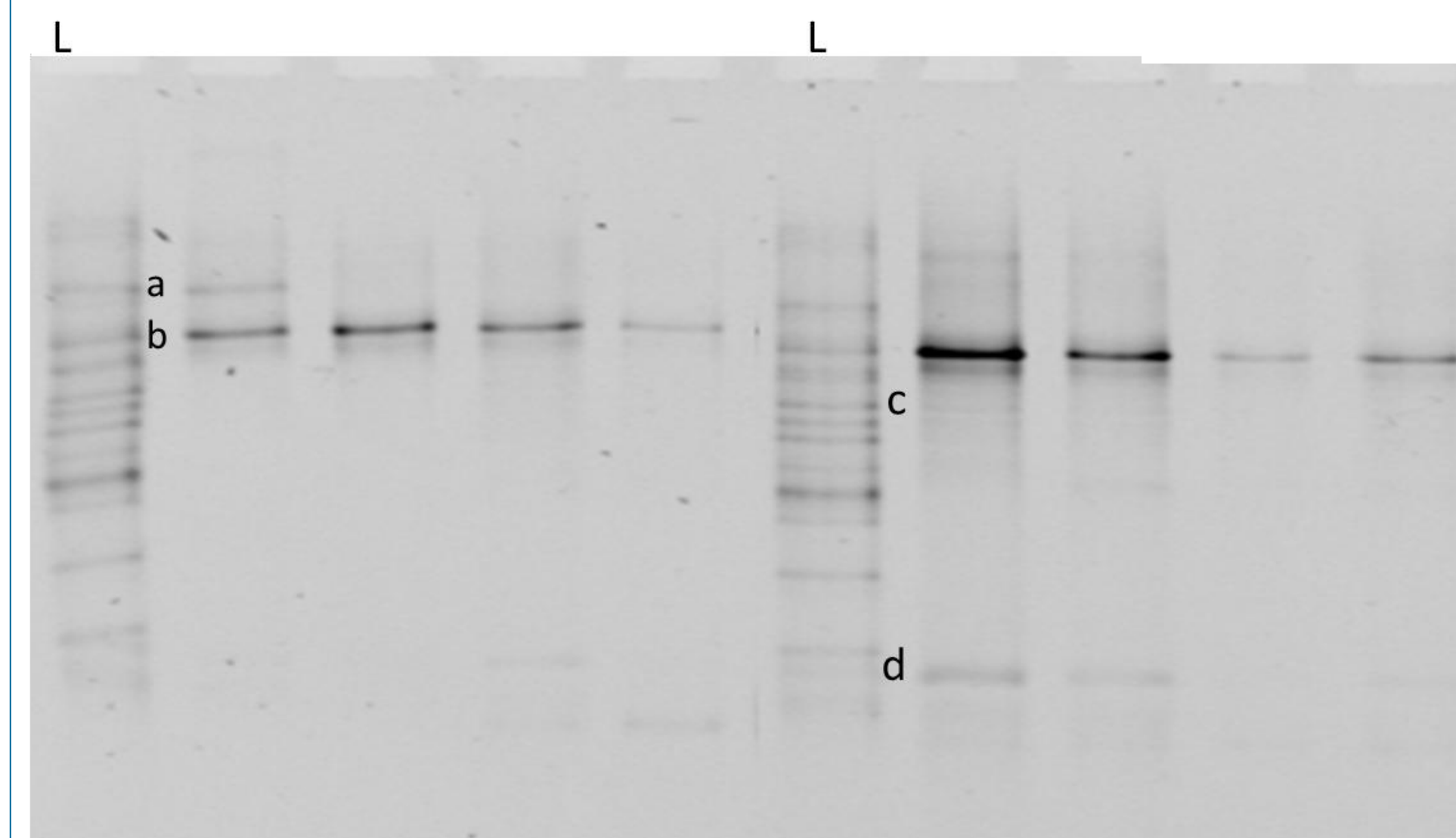
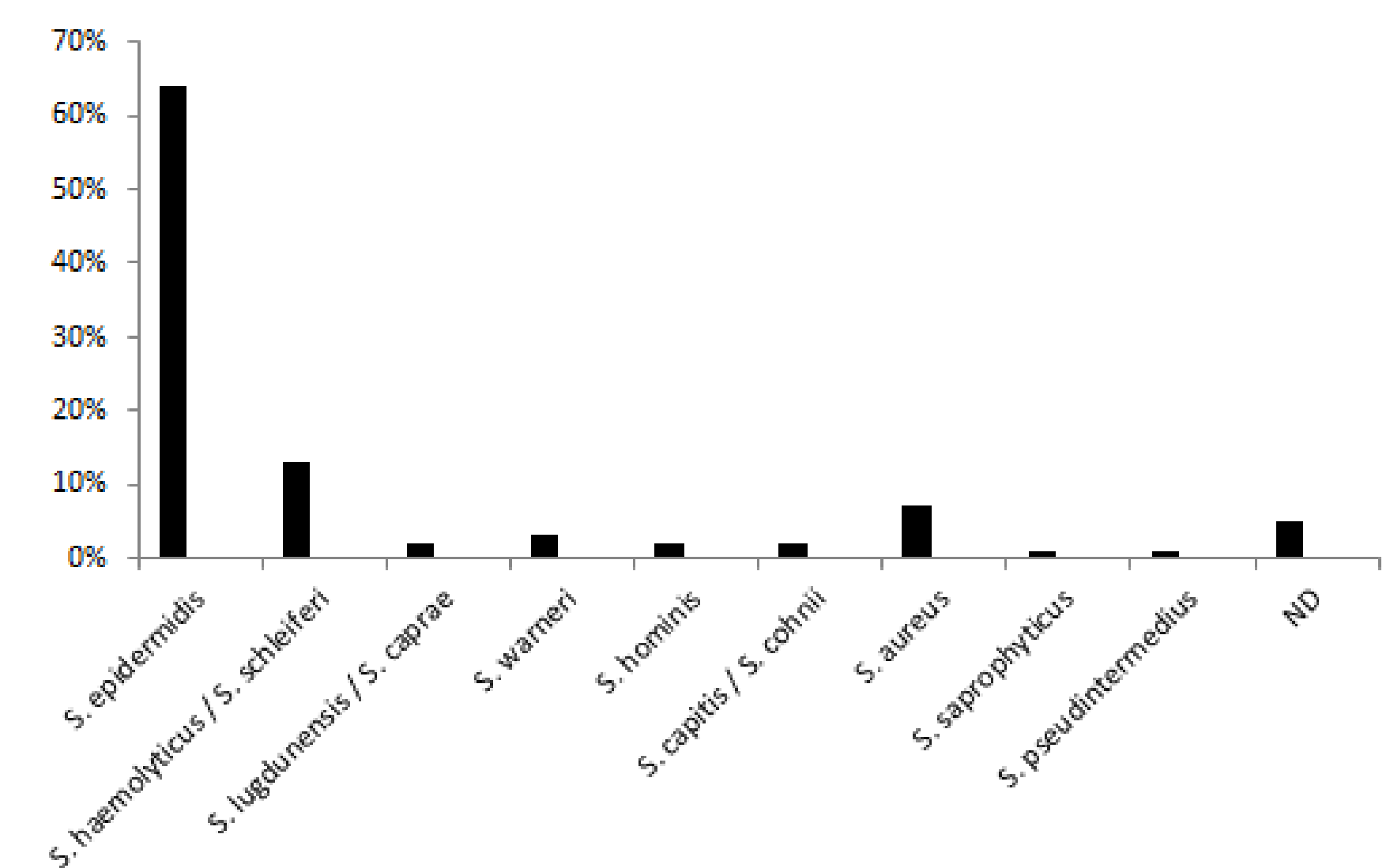
❖ Detection of CoNS DNA from 84/211 samples **39.8% of samples**

✓ 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.