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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

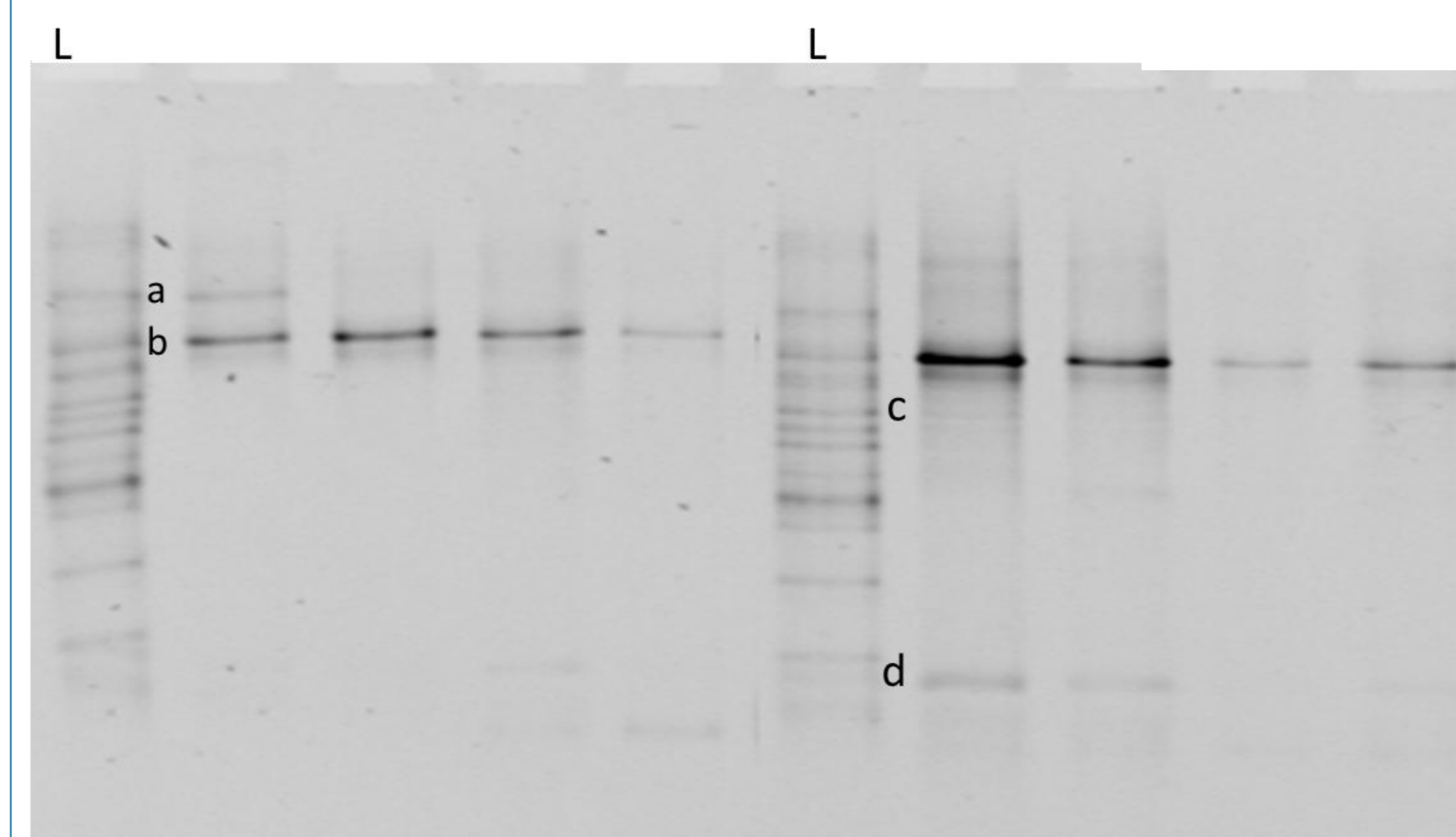
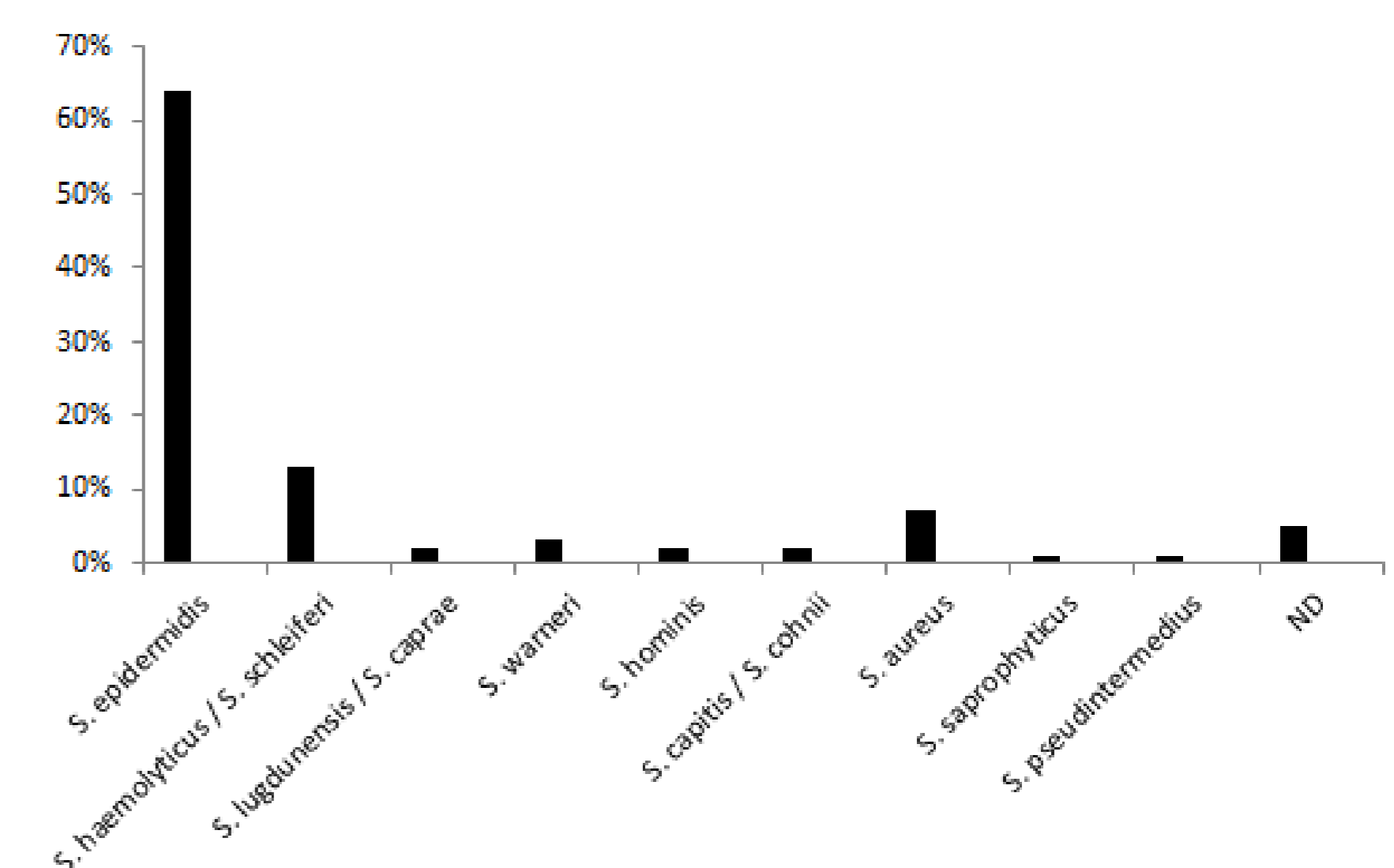
❖ *tuf*-PCR-TTGE after DNase digestion of free DNA

- ❖ 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.