



CLINICAL RESEARCH ARTICLE

Persistent microbial contamination of incubators despite disinfection

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BACKGROUND: In neonatal intensive care units (NICUs), hygiene and disinfection measures are pivotal to protect neonates from nosocomial infections. This study aimed to evaluate the efficacy of the classical incubators disinfection procedure and to follow-up neonates housed in the incubators for the development of late-onset sepsis (LOS).

METHODS: In a tertiary NICU, 20 incubators were monitored for bacterial contamination at three times: before disinfection, after disinfection, and 24 h after turning on and housing a new neonate. Clinical data of neonates housed in these incubators were retrieved from the medical records.

RESULTS: All 20 incubators were contaminated at the 3 times of the study, mainly on mattresses and balances. Coagulase-negative *Staphylococci*, *Enterococcus*, and *Bacillus*-resisted disinfection while enterobacteria and *Staphylococcus aureus* were eradicated. After 24 h, the bacterial colonisation was similar to the one observed before disinfection. The bacteria isolated on incubators were also found on the caregivers' hands. During the study, two preterm neonates developed a LOS involving a bacterial species that has been previously isolated in their incubator.

CONCLUSION: Pathogenic contaminants persist on incubators despite disinfection and represent a risk for subsequent infection in preterm neonates. Improvements are needed concerning both the disinfection process and incubator design.

Pediatric Research (2021) 90:1215–1220; <https://doi.org/10.1038/s41390-021-01407-8>

IMPACT:

- Procedures of disinfection that are usually recommended in NICUs do not allow for totally eradicating bacteria from incubators.
- Preterm neonates are housed in incubators colonised with potentially pathogenic bacteria.
- The control of nosocomial infections in NICUs requires further researches concerning mechanisms of bacterial persistence and ways to fight against environmental colonisation.

INTRODUCTION

In neonatal intensive care units (NICUs) late-onset sepsis (LOS) is a frequent issue associated with significant morbidity and mortality, especially in preterm very-low-birth-weight infants.¹ These infections are frequently nosocomial and the most frequent pathogens involved are coagulase-negative *Staphylococci* (CoNS), *Staphylococcus aureus*, and enterobacteria.¹

Previous studies have explored the possible sources and reservoirs of these pathogens within the NICU settings. On the one hand, caregivers could represent a possible source and/or vector of pathogens responsible for the contamination of neonates.² On the other hand, several authors have identified the environment and inert surfaces of the NICU as the reservoir of some pathogens. For example, the clone *S. capitis* NRCS-A, an emerging CoNS responsible for LOS worldwide has been retrieved from several surfaces in the NICU, especially on the incubators.^{3–5} Other bacteria including *S. aureus* and enterobacteria involved in nosocomial outbreaks have also been isolated on NICU equipment.^{6,7} These findings point out a possible failure in the process of disinfection.³

In that respect, the aim of the present study was to evaluate the efficacy and weak points of the procedure of incubator disinfection usually recommended on the basis of environmental samples in a tertiary NICU. The secondary objectives of this study were: (i) to follow-up neonates housed in the incubators of the study, especially for the development of LOS, (ii) to describe the bacterial epidemiology of incubator's contamination before and after disinfection, (iii) to investigate the hand skin colonisation among caregivers, and (iv) to determine other environmental sources of pathogens within the NICU.

METHODS**Study setting and methods of sampling**

The study was conducted in the Level III NICU of the Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, France during two consecutive weeks in June 2020. During this period, for each incubator that was disinfected five different sites were sampled (the rubber grommet, the left door handles, the temperature adjustment button, the mattress and the balance) using flocked

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Received: 2 October 2020 Revised: 19 January 2021 Accepted: 24 January 2021

Published online: 24 February 2021

swabs (ESwab, Copan®, United States) just before and just after the disinfection procedure. After 24 h during which the incubator was turned on and a neonate housed in (24H), the mattress and the rubber grommet were sampled again. In the same way, one heating table in the delivery room of this hospital was also sampled one time at five different sites (the mattress, the lateral door, the monitor, the timer button and the air-O₂ blender) since it could represent a potential reservoir for the early colonisation of neonates. This heating table was chosen because it was the one used in the delivery room for neonates that require hospitalisation in the NICU. Of note, this table is disinfected between each patient using Surfanius Premium (ANIOS, Lezennes, France) impregnated wipes. Finally, the disinfection room where incubators are disinfected between two patients was sampled one time at two sites: the computer keyboard and the surface where pieces of incubators are placed during drying.

Procedure of incubator disinfection

In the study NICU, two types of incubators are currently used: Giraffe™ (General Electrics Healthcare, Limonest, France) and SATIS+ (Médipréma, Tauxigny, France). In this setting, incubators are changed every 10 days or earlier if the neonate did not need it any more. Between two patients, the incubators are disinfected using a 20-min disinfectant immersion bath using a diluted solution of Surfanius Premium (containing *N*-(3-aminopropyl)-*N*-dodécylpropane-1,3-diamine 51 mg/g and didecylmethylammonium chloride 25 mg/g; final dilution 0.25%) (ANIOS, Lezennes, France). The protocol of disinfection of incubators is usual, according to the manufacturer recommendations. It consists in (i) an immersion of the removable parts of the incubator and (ii) decontamination of the frame and of the fragile parts with diluted Surfanius Premium impregnated wipes, as described elsewhere.³ The different incubator parts are then rinsed with filtered water and finally dried before the reassembly. When a neonate is housed in the incubator, the incubator is daily cleaned by nurses based on careful wipe disinfection using Surfanius Premium for the exterior of the incubator while the interior of the incubator (in contact with the neonate) is cleaned using sterile water.

Hand skin sampling among caregivers

During the study period, the hands of the caregivers involved in the process of disinfection were swabbed using sterile wet compresses twice each day, first after hand washing but before disinfection of the first incubator of the day, and second just after disinfection of the last incubator of the day, beforehand washing. Of note, in this NICU the same caregivers are involved either in incubator disinfection or in cares provided to neonates.

Bacterial culture of samples and bacterial identification

Contamination of a surface was defined by the presence of at least one bacterial strain belonging to a species that can be involved in LOS in neonates. That is why bacterial analyses aimed to identify the main pathogenic agents including CoNS (including *S. capitis* NRCS-A), *S. aureus*, Gram-negative bacteria and other bacteria of clinical relevance. Briefly, after 24 h of enrichment in brain heart infusion (BHI-T, BioMérieux, Marcy l'Etoile, France) at 37 °C, two agar plates were inoculated for each sample: one MRSA Brilliance 2 agar plate (Oxoid®, Versel, Germany) to isolate methicillin-resistant CoNS including *S. capitis* NRCS-A as previously described⁸ and one CPSE agar plate (Chrom ID® CPS® Elite, Biomérieux, Marcy l'Etoile, France) to isolate the other bacteria. Species identification was performed using matrix-assisted laser desorption-ionisation - time of flight mass spectrometry (MALDI-TOF MS) using the VITEK® MS system (BioMérieux, Marcy l'Etoile, France).

Collection of clinical data

Clinical data were retrospectively collected concerning the neonates housed in the incubators of the study after the

disinfection process. The clinical data were retrieved using the software ICCA (IntelliSpace Critical Care and Anesthesia, Philips®, Suresne, France) which is used to prospectively record medical information for patients in the study NICU. The following items were collected: gestational age and weight at birth, sex and small for gestational age status (according to revised Fenton curves)⁹ and age at the housing in the incubator. For each neonate, the occurrence of a LOS was searched. LOS was defined as the combination of (i) clinical signs of infection, whether hemodynamic, respiratory, or intestinal, and/or biological disorders (raised C-reactive protein and/or procalcitonin) in a patient older than three days of life; and (ii) a positive bacterial culture of blood sample; and (iii) antimicrobial treatment for more than five days or until the patient died.¹⁰ Positive culture of urinary sample and/or tracheobronchial suction and/or cerebrospinal fluid and/or operative site swabbing were also considered as infections if they were associated with clinical signs of infection and antimicrobial treatment more than five days or shorter if the patient died. For infected patients, additional items were collected: mode of delivery, premature membrane rupture more than 24 h before birth, intrapartum antimicrobial drugs administered to the mother, antimicrobial drugs administered to the neonate before the onset of sepsis, the delay between housing in the incubator and the onset of sepsis, age at sepsis, clinical signs, antibiotics administered for the sepsis.

Ethics statement

This study was approved by an ethical committee for biomedical research (Comité de Protection des Personnes Est I) under the number ID RCB 2020-A00818-31. Oral consent was obtained from the caregivers for the analysis of their hand skin samples. Written information was delivered to the parents of the infants involved in this study.

RESULTS

Efficacy of incubator disinfection

During the 2-weeks study period, 20 incubators were disinfected and were thus included in the analysis. All 20 incubators were contaminated both before and immediately after disinfection (details presented for each incubator in Supplemental Fig. 1). CoNS were the most frequently found bacteria at each time of the study with 100%, 90%, and 95% of incubators that were contaminated with CoNS before, after disinfection and at 24H, respectively (Fig. 1). Among the CoNS, *S. epidermidis* was the most frequent species followed by *S. capitis* and *S. haemolyticus*. *S. aureus* and enterobacteria (in particular *Enterobacter*, *Escherichia*, and *Klebsiella* strains) were identified before disinfection but were eradicated from the sites of sampling after disinfection. However, *S. aureus* and enterobacteria were isolated again in, respectively, 15% and 30% of the 20 incubators at 24H. *E. faecalis* and *Bacillus* were also isolated at the 3 time-points of the study even if disinfection was associated with a decreased frequency of isolation.

Concerning the different parts of the incubators, the mattress and the balance remained the most frequently contaminated sites after the disinfection procedure with, respectively, 100% and 90% of positive samples just after disinfection (Fig. 2a). For all sampled sites, CoNS were once again the most frequently isolated bacteria (Fig. 2b). In particular, *S. capitis* NRCS-A was the most frequent bacteria isolated on the mattress both before disinfection (24% of all isolates) and at 24H (23% of all isolates). Of note, *S. aureus* was isolated before disinfection on the grommet, the temperature adjustment button and the mattress but was absent elsewhere (Fig. 2b). At 24H, the number of contaminated sites increased in comparison with the results just after disinfection and the repartition of the bacterial species tended to the one observed before disinfection (Fig. 2).

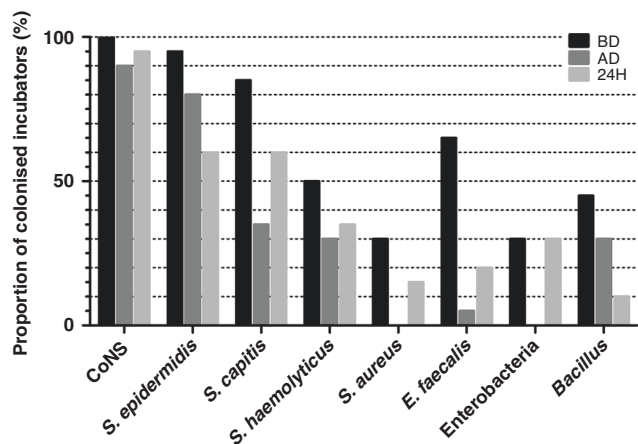


Fig. 1 Bacterial species isolated on the 20 incubators. Percentage of incubators that were colonised with each bacterial species or group among the 20 incubators of the study at the different times of the study: before disinfection (BD), after disinfection (AD), and after 24 h during which the incubator was turned on and a neonate housed in (24H). Details of the species identified on incubators are presented in Supplemental Table 1.

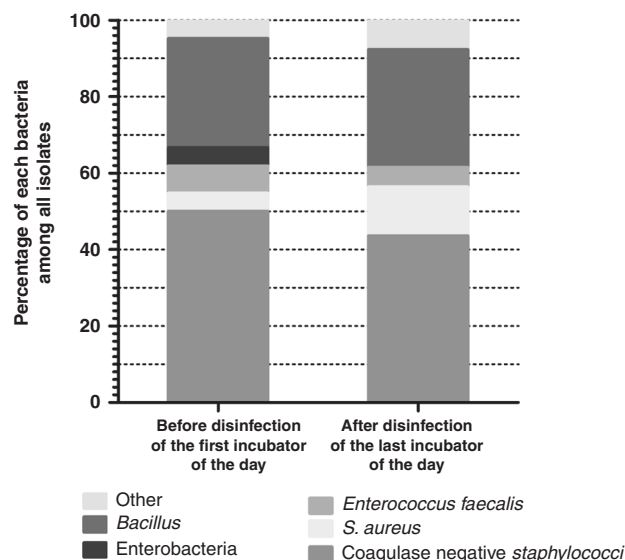


Fig. 3 Bacteria isolated from the hand samples of the caregivers who disinfected incubators depending on the time of sampling. The hands of the caregivers were sampled before disinfection of the first incubator of the day and after disinfection of the last incubator of the day. The percentage of each bacterial group or species was calculated compared to the total number of isolates. Each species or group is represented by a specific colour. Details of the species identified on the hand samples of caregivers are presented in Supplemental Table 1.

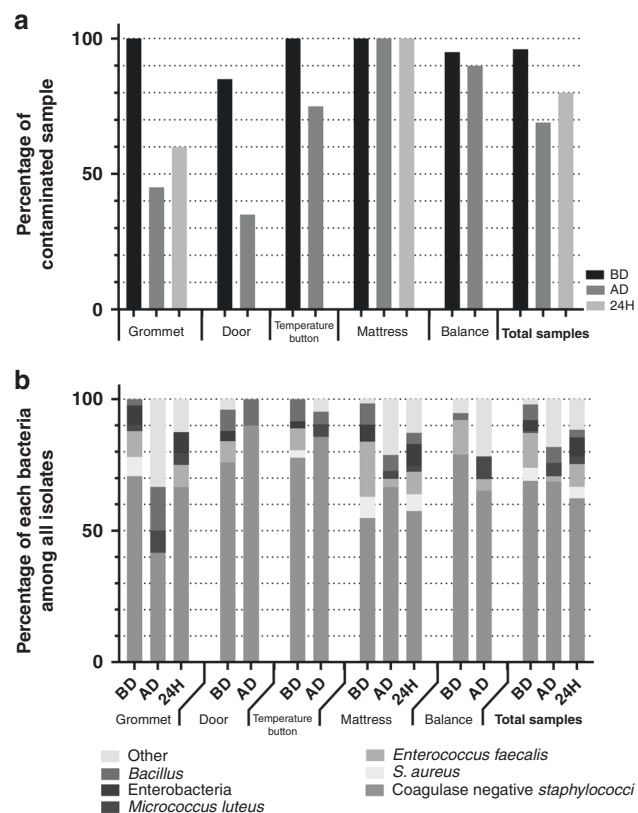


Fig. 2 Contamination of the different incubator sites depending on the time of sampling. Each site was sampled before disinfection (BD) and after disinfection (AD). The grommet and the mattress were also sampled after 24 h during which the incubator was turned on and a neonate housed in (24H). **a** Percentage of total positive samples BD, AD, and 24H includes the 5 sites of the 20 incubators. **b** Percentage of bacteria isolated on the different incubator parts. For each site, the percentage of each bacterial group or species was calculated compared to the total number of isolates. Each species or group is represented by a specific colour. Details of the species identified on incubators are presented in Supplemental Table 1.

Other environmental reservoirs of bacteria

To detect other environmental putative reservoirs in the NICU, additional samples were performed. Inside the disinfection room, *S. epidermidis* was isolated on the computer keyboard and on the bench where pieces of incubators are placed during drying. Moreover, *Bacillus cereus* was isolated on the bench. In the delivery room, we identified *S. hominis* on the air-CO₂ blender and *Micrococcus luteus* on the lateral door and on the timer button.

Hand colonisation among caregivers

During the study period, seven different caregivers were involved in the process of incubator disinfection and a total of 22 hand skin samples was obtained. The hand swabbing from caregivers showed a predominance of CoNS, *Bacillus*, *Enterococcus faecalis*, enterobacteria, and *S. aureus* before disinfection of the first incubator of the day (Fig. 3). At the end of the day, the diversity and repartition of bacteria remained similar, except for an increase in the frequency of isolation of *S. aureus* and the absence of enterobacteria. Even if CoNS were frequently isolated, *S. capitis* was isolated only on two caregivers' hand skin samples at the end of the day.

LOS occurrence during the study period

The 20 neonates housed in the incubators of the study were preterm (median gestational age 28 weeks, extremes 24–34 weeks) with a median birth weight of 1020 g (extremes 530–2075 g). Nine (45%) were small for gestational age. They were housed in one of the study incubators at a median of 13 days of life (extremes 0–43 days). Among these 20 patients, three extreme preterm patients developed a LOS during their housing in the incubator. The characteristics of these three neonates are presented in Table 1. Interestingly, for two of these three infected patients, the bacterial species involved in the sepsis has been also previously isolated in the housing incubator. The three neonates recovered from the infection.

Table 1. Characteristics of the three neonates who have developed sepsis when they were housed in one incubator of the study.

| | Patient 1 | Patient 2 | Patient 3 |
|---|-------------------------------|---|---|
| No. of incubator | 7 | 8 | 12 |
| Perinatal data | | | |
| Premature rupture of membranes | no | yes | yes |
| Antibiotics administered to the mother | No | Yes | Yes |
| Delivery mode | Caesarean section | Vaginal | Caesarean section |
| Gestational age at birth (weeks) | 27 | 24 | 26 |
| Birth weight (g) | 900 | 595 | 990 |
| Small for gestational age | No | No | No |
| Antibiotics administered to the neonate before sepsis | No | Yes | Yes |
| | | Penicillin G and amikacin during 2 days for suspicion of early-onset sepsis | Penicillin G and amikacin during 2 days for suspicion of early-onset sepsis |
| Sepsis | | | |
| Age at the sepsis (days) | 44 | 27 | 18 |
| Clinical signs of sepsis | Rectal bleeding | Respiratory instability with increased respiratory support and O ₂ requirement | Hemodynamic failure, respiratory instability, and a bowel perforation |
| Biological disorders | Neutropenia (1.1 G/L) | Increased CRP (167 mg/L) | Increased CRP (222 mg/L) and PCT (96 µg/mL) |
| Site of bacterial isolation | Blood culture | Tracheobronchial suction | Blood culture and peritoneal fluid sampled during surgery |
| Bacterial species involved in the sepsis | <i>Staphylococcus hominis</i> | <i>Staphylococcus haemolyticus</i> | <i>Enterococcus faecalis</i> |
| Antibiotics administered for the sepsis | Vancomycin during 7 days | Linezolid during 10 days | Amoxicillin during 16 days |
| Death | No | No | No |
| Incubator | | | |
| Site of incubator colonisation with the same bacterial species | Mattress | Left door | No identification |
| Time of the study of isolation of this bacteria | Just after disinfection | Just after disinfection | No identification |
| Delay between housing in the incubator and onset of sepsis (days) | 9 | 7 | 1 |

DISCUSSION

The present work highlights the partial ineffectiveness of the disinfection process of incubators that are currently performed in most NICUs as well as the potential consequences and risks for hospitalised neonates.

Several hypotheses could explain this partial failure in incubators' disinfection. First, incubators are composed of many delicate parts and recesses that cannot be immersed in the disinfectant bath and that have to be manually disinfected with impregnated wipes. Two major examples are the mattress and the balance that were the most contaminated parts of the incubators after disinfection in our study and previous ones.³ Due to its tissue composition and the presence of seams, the mattress is probably a primary reservoir for bacteria. In that regard, a recent study conducted in a NICU setting showed that among 42 incubator mattresses tested, all were contaminated both on the cover and in the foam.⁶ Second, bacteria could resist the disinfection and persist on incubators because of their capacity to produce biofilm. Previous studies have demonstrated that bacteria embedded in biofilm matrix are less sensitive to disinfectant than planktonic ones^{11,12} and are able to persist in NICU environment.^{2,13} Third, the failure of disinfection could be related to a relative bacterial tolerance to disinfectants compounds. In particular resistance mechanisms against quaternary ammoniums have already been

reported in NICU isolates.¹⁴ Finally, in light of our findings showing a wide bacterial diversity on incubators after disinfection, we have to consider a possible early recolonisation from the environment and/or caregivers. This hypothesis is reinforced by the isolation of several pathogenic species both in staff hands and incubators samples, especially CoNS that are part of the normal skin commensal microbiota. The transient carrying of CoNS pathogenic strains in caregivers has already been highlighted.³ Hira et al.² demonstrated both a higher prevalence of multidrug-resistant CoNS on the hands of caregivers in comparison of the general population, and a 90% genetic correlation between CoNS strains isolated from caregivers and those responsible for LOS in a NICU. Thereby, we hypothesised that in our study, CoNS isolated on incubators (in particular methicillin-resistant strains, growing on MRSA Brilliance 2 agar plates) may come from caregivers. This reminds the importance of the strict respect of standard hygiene measures in order to avoid cross-transmission not only during patient cares but also when touching medical devices. One limitation of our study is the absence of data concerning the hand skin colonisation of parents, who can also be involved in the steps of contamination of incubators and/or neonates. The improvement of hand hygiene of all people touching newborns and/or environmental NICU surfaces (caregivers, parents and visitors) has already been reported as one of the most important strategies to

avoid cross-transmission and to decrease infection risks for patients in NICUs.^{15,16}

In our study along with other ones, reporting such defective disinfection is of major concerns since it reflects a supplementary LOS risk factor for preterm neonates who are particularly vulnerable to infections due to their immature immune system and skin barrier.¹ In our study, 2 of the 20 patients developed sepsis involving a bacterial species previously isolated in the incubator. Because of the absence of strain molecular analysis and the lack of data concerning the previous microbiota composition of these infected patients, we can neither attest nor exclude the causality between incubator contamination and patient subsequent infection. Nonetheless, the major part of bacteria isolated at the different times of our study are known as potential pathogens responsible for neonatal sepsis and have already been isolated from NICU incubators in previous studies.^{3,6,17} In particular, even if CoNS are part of the normal environmental colonisers, they have to be considered since they are frequently involved in LOS. The link between environmental colonisation (especially incubators) and outbreak in NICUs has previously been demonstrated involving sometimes virulent and multidrug-resistant strains.^{6,17–19} Interestingly here we highlight that while the disinfection process was effective for common pathogens (*S. aureus*, enterobacteria) immediately after disinfection, those pathogens quickly recolonised. This suggests that in addition to an improved process of incubator disinfection, it is necessary to perform a more frequent/in-depth cleaning of incubators during housing infants.

An improvement in incubator disinfection in NICUs worldwide is urgently required given that some authors previously reported its benefits in controlling outbreaks and in preventing nosocomial infections in NICU settings.^{6,19–21} An interesting method of disinfection could be the steam technology which consists in spraying steam at high temperature (130–150 °C) and high pressure (4–6 bars) to reach the recesses that are difficult to access with the classical chemical method.²¹ It has been shown to significantly reduce the bacterial load on incubators in a previous study²¹ and to be effective in the fight against NICU outbreaks involving either *S. capitis* NRCS-A or vancomycin-resistant *Enterococci*.^{19,20} Moreover it is more cost-efficient and less water consuming.^{21,22} Finally, it avoids exposure of neonates and staff to toxic and potentially allergenic chemicals able to adsorb on incubators surfaces.^{6,20,21}

However, steam could not be recommended for mattress disinfection according to Cadot et al.⁶ who suggested that formation of moistures on the inner layers was related to the use of this method. Another approach to improve the efficacy of incubator disinfection could be an in-depth consideration about incubators' design with fewer recesses and maybe single-use mattresses or mattress protective covers. To this end, further studies should be conducted to help in the choice of the materials constituting the different incubators' parts to select the ones preventing microbial adherence. The combination with immobilised non-toxic antimicrobial molecules might also contribute to inhibit microbial presence in these surfaces as suggested in recent studies.^{23–25}

Our work presents several limits. First of all, only five sites were sampled for each incubator which is less than other studies²⁶ and our method of analysis only provides qualitative data. However, these sites were chosen because they are either the most frequently touched or because they have previously been identified as bacterial reservoirs within the incubators.^{3,6,26} Second, the discrepancy that may appear when comparing the bacteria isolated on the same incubator at the three times of the study suggests a limit of sensitivity in our protocol of sampling and bacterial identification. Third, since CoNS are part of the normal skin and environmental colonisers, our study does not allow the identification of potentially pathogenic strains among all CoNS. However, considering all CoNS isolated here is the most rigorous method to avoid underestimation of risks for neonates.

In a future study, a better characterisation of the CoNS strains coming from incubators, caregivers, parents and the general population would be useful to better understand the origin and ways of neonatal contamination with CoNS in NICUs. Finally, the absence of molecular analysis of the strains prevents us to attest the link between the strains involved in infections and those identified from incubators.

CONCLUSIONS

The process of decontamination as it is currently recommended is not sufficient to eradicate bacterial colonisation from incubators and in particular pathogenic strains. Incubators could thereby represent an important reservoir of the pathogen. Incubator disinfection is a milestone in the fight against nosocomial contamination and infection. Future research should focus on re-evaluating the strategy of incubators disinfection as well as incubators materials choice and design.

ACKNOWLEDGEMENTS

This work was supported by ANR (Agence Nationale de la Recherche) project NeoSCap [grant number ANR 19-CE17-0004-01].

AUTHOR CONTRIBUTIONS

Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data: M.C., M.R., J.T., and M.B. Drafting the article or revising it critically for important intellectual content: M.C., M.B., A.T., and O.C. Final approval of the version to be published: all authors.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41390-021-01407-8>.

Competing interests: The authors declare no competing interests.

Consent statement: Written information was delivered to the parents of the infants involved in this study. Oral consent was obtained from the caregivers for the analysis of their hand skin samples.

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