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Research paper

# "When no-one's looking," the application of lung recruitment and normal saline instillation with paediatric endotracheal suction: An exploratory study of nursing practice

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ABSTRACT

*Background:* The complex nature of the Paediatric Intensive Care Unit (PICU) patient requires the bedside nurse to make rapid, complex decisions regarding endotracheal suction (ETS) interventions. It is not understood what influences nurses' decision making in the context of ETS, however, the actions of the clinician have a direct impact on the efficacy of the ETS event and patient outcomes.
*Objectives:* To explore and describe the use of normal saline instillation and lung recruitment with paediatric ETS in a cohort of Australian nurses, and to identify factors that influence normal saline use with ETS.
*Methods:* A descriptive, exploratory study. An evidence-based practice model formed the conceptual basis for the study. Semi-structured interviews were conducted with 12 nurses from an Australian tertiary referral paediatric intensive care unit. Audiotaped interviews were transcribed. Inductive thematic analysis was used to code and analyse the interview data and identify themes.
*Findings:* Data analysis revealed three themes: patient's clinical presentation, clinician judgement and unit practice norms.
*Conclusions:* Variability in nurses ETS practice was marked. In the absence of evidence based clinical guidelines, nurses relied on knowledge derived from clinical experience and the local setting to guide NSI and LR intervention decisions. Participants reported uncertainty regarding ETS best practice and perceived the lack of research evidence as a barrier to making informed clinical decisions at the bedside. Rigorous research evaluating the safety and efficacy of NSI and LR with ETS is urgently required for patient care; however PICU nurses rely on multiple sources of evidence to inform ETS practice decision.


## 1. Introduction

Annually, around 10,000 Australian children are admitted to a paediatric intensive care unit (PICU).[1] Fifty percent of these children require endotracheal intubation and mechanical ventilation. Insertion of the endotracheal tube (ETT) to support respiration inhibits mucociliary clearance mechanisms, and endotracheal suction (ETS) becomes a vital intervention to maintain ETT patency.[2] ETS is considered routine care by PICU clinicians, with reported

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frequencies of more than 40 times per PICU admission, dependent on disease severity and length of ventilation.[3] Despite the importance of ETS to maintain the airway, complications such as infection and hypoxia are common.[4,5] These complications are associated with increased ventilator days and in the case of ventilator associated pneumonia, increased morbidity or mortality.[5–7]

There is no international consensus on paediatric ETS best practice. Current practice includes the addition of interventions such as normal saline instillation (NSI) prior to ETS and lung recruitment (LR) following ETS. The stated goal of NSI is to facilitate secretion removal by mixing or diluting airway mucous.[2,8] However, the safety and efficacy of NSI in paediatric practice is widely debated with some studies reporting a negative association between NSI and oxygenation.[9–11] These studies however had small sample sizes that were unlikely to statistically detect differences between groups and high risk of bias. Further, this argument fails to account for the deleterious effect ETS has on lung volume. ETS causes rapid, inhomogeneous, derecruitment (end-expiratory collapse) of alveoli.[4] LR applied after ETS may improve oxygenation by providing positive airway pressure above the critical opening pressure of the airspace to recruit (reopen) collapsed alveoli.[4,12,13] This is of particular interest in children with acutely injured lungs which are characterised by fluid filled, collapsed alveoli which does not participate in gas exchange.[14] Although regularly applied in the PICU, ETS interventions including NSI and LR are not based on compelling clinical trial data.

Clinical and best practice guidelines help reduce variation in practice and inform clinical decisions at the bedside; however, such practice documents rely on quality, relevant evidence to inform recommendations. There are no published studies which definitively test the safety and efficacy of NSI or LR in the PICU population. In the absence of NSI and LR trial data and clinical guidelines, it is important to understand what influences nurses' NSI and LR practice decisions. The primary objective of this study was to explore PICU nurses' experiences with NSI and LR in association with ETS. Further, we explored the clinical indicators that influence nurses to apply NSI with ETS. We will provide exploratory evidence from PICU nurses on NSI and LR practices with paediatric ETS, which will provide the basis for further examination of ETS methods in PICU.

## 2. Methods

### 2.1. Qualitative approach

A descriptive, exploratory study of nurses' experiences of NSI and LR with ETS was conducted using semistructured, face-to-face interviews. The study is reported using the Standards for Reporting Qualitative Research.[15]

An evidence-based practice model[16] formed the conceptual basis for the study. A fundamental difficulty for clinicians is that many PICU interventions like NSI and LR lack evidence.[17–19] In the absence of systematic scientific enquiry, nurses rely on other sources of evidence to inform practice.[20] The evidence-based practice model[16] suggests knowledge which informs practice is derived from four key sources of evidence: research evidence, clinical experience, patient experience, and local context information. The clinician may draw on multiple sources of evidence to inform practice decisions; however, the way clinicians apply this evidence to inform practice whilst considering individual patient needs has yet to be explored in relation to NSI and LR in the PICU setting.

### 2.2. Researcher characteristics and reflexivity

Reflexivity is an important consideration in qualitative inquiry as it emphasises the value of self-awareness of one's own perspective. The aim of undertaking ongoing reflexive exercises is to prevent researcher bias from diminishing the credibility and the rigour of the methods.[21] The research team comprised four females including: one clinical nurse (PhD candidate and chief investigator [CI]); a PICU nurse researcher (PhD), a professor of critical care nursing (PhD), and a professor of nursing (PhD). The clinician–researcher relationship was one that allowed the researcher to have a deep understanding of the participants' situations however the established professional relationship may have inhibited certain points of discussion. Participants were aware of the CI's reason for conducting the research.

### 2.3. Setting

The single centre study took place at tertiary referral PICU in Brisbane, Australia. Interviews were conducted in an office outside the clinical environment. Only the participant and the researcher were present during interviews.

### 2.4. Participant selection

A purposive sampling approach was used to recruit participants. Purposeful sampling is widely used in qualitative research and involved the identification of PICU nurses who had experience with the use of NSI and LR in the site PICU.[22] Participants were invited to participate in the study upon satisfying the inclusion criteria: (i) permanently employed PICU nurse at the site and (ii) mechanical ventilator competency. An email was sent to staff advising them of the study and inviting participation. Contact details of the CI were provided. Sample size was not defined a priori, and data were gathered until saturation was achieved, that is, when no new information was being identified in interview data.[23,24]

### 2.5. Ethical considerations

Ethics approval was obtained from the hospital and university human research ethics committee (HREC/16/QRCH/374; 2017/065). Participants were provided with verbal and written information detailing the purpose of the study, right to withdraw consent, assurance of confidentiality, and CI contact details. Written informed consent was obtained before the interview which was audio recorded. The confidentiality of participants was maintained throughout the study. Recorded responses were de-identified, and audio transcripts did not contain identifiable information.

### 2.6. Data collection methods and instruments

Interviews were conducted in February 2017. To ensure consistency, an interview guide was used, and participants were asked identical open-ended questions.[23,25] The interviewing method included a variation of both descriptive and structured questions.[26] Questions were based on key areas of interest related to paediatric ETS, NSI, and LR as identified in the literature and included: (i) clinical indicators for NSI use; (ii) rationale for NSI and LR use or non-use with ETS; and (iii) influences on practice.[2,3,9,27–29] Follow-up questions and prompts were adapted based on participant responses during the interview; this allowed a more individualised approach[30] and full exploration of participant experience and viewpoint. Some forced response questions were included to explore specific factors in relation to NSI use (for example interviewee's clinical experience, habit, unit practice, and research evidence). At the end of the interview nurses were asked to rate factors that influence their use of NSI with ETS. A Likert scale of 1–5 (least influential to most influential) was used to determine the level of influence. All interviews were professionally transcribed for accuracy.[21] Field notes were made at the conclusion of the interview. Participants were recruited until no new relevant information was being obtained from new participants.[11] Interview duration was 15 min.

### 2.7. Data analysis

Inductive thematic analysis was used to code and analyse the interview data and identify themes as interviews were conducted. Analysis was data driven and as per Braun and Clarke's six phases of thematic analysis: (i) familiarising with data; (ii) generating initial codes; (iii) searching for themes; (iv) reviewing themes; (v) defining and naming themes; and (vi) producing the report.[31] Following full transcription, interview data were coded by the first author, the CI. Initial codes were generated using line-by-line coding (facilitating an audit trail) and a process of writing and grouping like ideas and patterns (Table 1). Codes-informed concept formation and themes were identified. Themes were reviewed and defined with continued reference to codes and raw data via discussion with the project team.[23]

### 2.8. Reliability

In a reflexivity exercise, extracted themes were presented to all interviewees. This provided a degree of trustworthiness and confirmability of findings. Authenticity was addressed through fairness (all PICU nurses who met eligibility criteria were invited to participate in the project). Further the investigator maintained a degree of reflective awareness of preconceptions and expectations throughout the data collection period.[32]

## 3. Results

### 3.1. Participant characteristics

In total 12 nurses were interviewed. Participants' characteristics are reported in Table 2. Participants were aged between 20 and 40 years, 11 were female. The majority of participants (n = 11) had less than 10 years PICU experience, and more than half (n = 7) had a postgraduate nursing qualification.

### 3.2. Factors that influence nurses' use of NSI

Factors that influenced the nurses' use of NSI are listed in Table 3. Nurses reported thick secretions as the top influencing factor with a total score of 59 out of 60. Clinical experience (48/60) and enhanced secretion yield (40/60) were the second and third highest scoring factors, respectively.

### 3.3. Themes

Analysis of interview data revealed three main themes: (i) patient's clinical presentation; (ii) clinician judgement; and (iii) unit practice norms. The thematic map is presented in Fig. 1.

#### 3.3.1. Patient's clinical presentation

Two subthemes were apparent within this theme: lung pathology and response to ETS. Nurses perceived children with respiratory disease or "sick lungs" as more likely to need NSI or LR with ETS.

> *"I would tend to use it more in those patients that have an underlying lung pathology or some kind of respiratory reason for their admission" (S01).*

**Table 1**
Example of line by line coding and concept formation.

| Transcribed data | Concepts |
|---|---|
| I would tend to use it with patients that have got thick secretions. | Thick secretions |
| Anyone that might be difficult to bag or sounds like they've got a lot down their chest. Those patients with quite concrete chests difficult to ventilate. What am I trying to say? Stiff lungs. That's what I'm trying to say. Those that you can hear the secretions on. | Sick chest<br>Stiff lungs |
| I don't use it with every child that I bag and suck. | Not always required |

**Table 2**
Participant characteristics.

| Demographic data | Number of participants (n = 12) |
|---|---|
| **Identifying gender** | |
| Female | 11 |
| Male | 1 |
| **Age (yrs)** | |
| 20–30 | 5 |
| 31–40 | 7 |
| **Education** | |
| Bachelor in nursing | 5 |
| Postgraduate certificate in nursing | 5 |
| Masters of nursing | 2 |
| **Nursing experience (yrs)** | |
| ≤5 | 3 |
| 6–10 | 7 |
| 10–20 | 2 |
| **PICU experience (yrs)** | |
| ≤5 | 7 |
| 6–10 | 4 |
| 11–20 | 1 |
| **PICU position** | |
| Registered nurse | 7 |
| Clinical nurse | 5 |

Yrs = years; PICU = paediatric intensive care unit.
Clinical nurse: An experienced registered nurse who has usually undertaken advanced qualifications or training in paediatric intensive care.

**Table 3**
Factors that influence nurses' use of NSI with ETS.

| Factors | Score (range 1–5) median (IQR) |
|---|---|
| Thick secretions | 5 (5–5) |
| Clinical experience | 4 (3.75–5) |
| Enhance secretion yield | 3 (3–4.25) |
| No secretions without NSI | 2.5 (1–4) |
| Education | 2.5 (1–4) |
| Research evidence | 2.5 (1–4) |
| Lubricate catheter | 2 (2–3) |
| Unit practice | 1.5 (1–2.25) |
| Co-worker practice | 1 (1–3) |
| Stimulate cough | 1 (1–2) |
| Habit | 1 (1–2.25) |

ETS = endotracheal suction; IQR = interquartile range; NSI = normal saline instillation.

> *"If it's a really healthy child that has just been intubated for the sake of the procedure that they've had. I don't use it then" (S10).*

The majority of nurses stated they apply NSI based on the patient's lung pathology and consistency of respiratory secretions. All nurses discussed using NSI with ETS to loosen thick secretions and aid in their removal. "*It* [NSI] *helps with bringing up the secretions when the secretions are quite thick" (S08).*

One participant also talked about the perceived value of NSI with ETS for breaking up mucous plugs which may obstruct the ETT

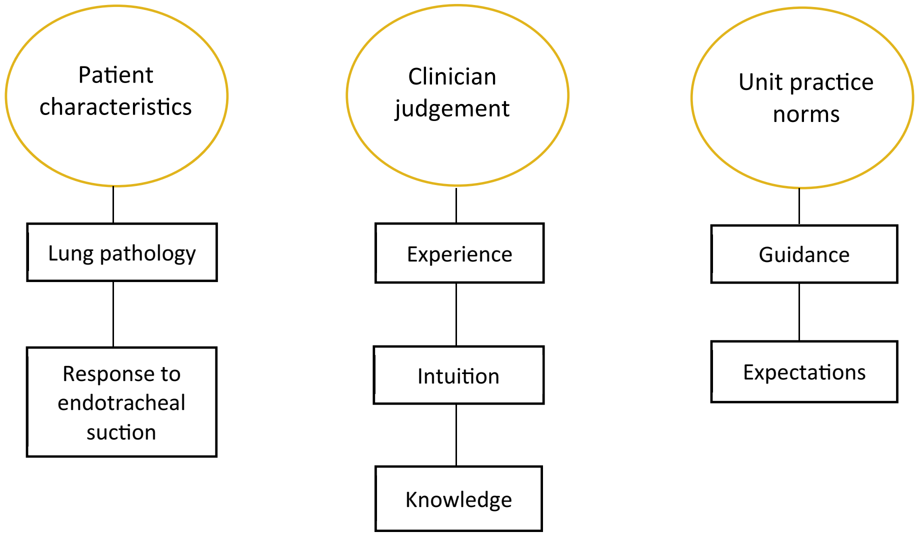


**Fig. 1.** Final thematic map.

"*if there was a plug or resistance … at the end of the ETT, instilling a liquid to help breakup a plug or loosen secretions in the ETT* [is helpful]" (S09).

Further, nurses identified children with underlying lung pathology as more likely to have a poor response to the ETS procedure, and they varied their practice accordingly. This was evident as nurses frequently described children with respiratory disease or sick lungs as more likely to decompensate after ETS "*these kids don't respond well to being bagged and sucked*" (S01). The nurses who applied LR (approximately one third) stated it would often depend on the patient's response to the previous ETS that determined their application of LR in subsequent ETS events "*how they behave* [post suction], *how they performed with the last suction*" (S01). Nurses stated these children are often "*PEEP [Positive End Expiratory Pressure] dependent,*" and nurses described how they had to take the initiative to resolve ETS complications such as post ETS desaturation events by using LR:

> *"You can see as soon as you put them back on [the mechanical ventilator] that their saturation starts to fall, I would re-recruit in discussion with the medical staff…when the lungs are sick they require high pressures to reinflate. That's (the) benefit, reinflating the alveoli" (S09).*

### 3.3.2. Clinician judgement

This theme incorporated three subthemes: experience, instinct, and knowledge. Nurses described the importance of experience by stating "*Every suction event is different, it's experience and practice*" (S01) and "*It's just experience*" (S02). Nurses perceived a lack of experience as problematic and expressed this through statements like "*Certainly the junior staff I don't think would ever do it* [LR] *unless you specifically told them*" (S01).

The second subtheme evident in the data suggested that nurses rely on instinct to guide ETS practice decisions. Statements such as "*It's all a bit of a gut feel thing*" (S01) and "*It is just me. It's a gut feeling … this is what I am going to do. People do what they feel is right*" (S05) mirrors most of the nurses' comments grouped in this theme.

The final subtheme knowledge captured nurses' understanding of the practical aspects of the interventions NSI and LR and the pathophysiological rationale behind the treatments. The data captured highlighted nurses' uncertainty of the effect of NSI and concern regarding the distribution of saline in the lungs, if the NSI was not aspirated in its entirety with the suction. Nurses described this using statements like "*We don't know what saline does to the lungs*" S01; "*I don't put too much saline in because we really don't know how much we can suck out*" (S07) and "*I don't know if there is any effect actually on the lung itself. I think it just helps the secretions in terms of a good or bad effect. I don't know, actually*" (S04).

Nurses also described a lack of knowledge in relation to LR and an uncertainty in how to apply this treatment using statements such as "*I apply it when no one's looking*" (S01), "*It might not be the best manoeuvre for them*" (S12), and "*I probably don't do it right*" (S10).

### 3.3.3. Unit practice norms

Two subthemes were contained within this overarching theme: guidance and expectations. A sense of uncertainty was described by all nurses who perceived there to be limited guidance to inform ETS practices in the context of NSI and LR. Nurses used words such as *non-standardised* and *habitual* to describe NSI and LR practice and *unsure* and *unclear* to describe unit ETS expectations. Nurses across all levels of experience and position (experienced nurse and registered nurse) suggested this was not optimal. Nurses identified a lack of research as contributing to this issue with statements like "*I don't have research or any evidence other than my own practise*" (S06) were common in this theme. Another nurse commented "*… there's quite a few things in this job that you don't actually have evidence for but you know that it works and it's an accepted behaviour rather than evidence based, as rightly or wrongly as that is*" (S03). Some nurses described uncertainty when faced with a lack of evidence regarding safety and benefit of NSI with ETS, one participant commented "*There was not a lot of evidence to suggest it was actually of benefit. I don't know if it was a harmful intervention*" (S10).

A lack of NSI and LR protocols and awareness of protocols was identified by the nurses. For example, nurses commented "*We don't have policy or protocol,*" (S02) "*there's nothing concrete, it seems to be a lot of opinion-based,*" (S09) and "*we don't have structure around what we should be doing, so it's hard to do evidence based nursing*" (S10). Nurses were motivated to explore how NSI and LR could support them but felt restricted by a lack of guidance and protocols.

This theme also captured nurses' perceptions of unit expectations of ETS nursing practice in the context of NSI and LR. Statements such as "*that's unit practice,*" "*it's the unit culture,*" and "*it's what you see your colleagues doing*" were common amongst participants when describing NSI use with ETS. Several nurses commented on the expectation to use NSI routinely "*it was belted into my head, that's the accepted practice … but it shouldn't be a routine" (S1) and "the nurse will be like, oh we'll just use saline anyway. You're like well, there's no clinical indication to, but it seems to be a bit routine now that we're using it" (S12).*

The majority of nurses expressed uncertainty as to whose role it was to perform LR after ETS. Statements such as*: "whether we should do it … I don't know?" (S02); "I would use it if it was—if it had been medically instructed;" (S09) and "I don't think our nursing population has the skill set" (S01)* captured nurses' hesitancy to perform LR. For both NSI and LR interventions, nurses perceived a lack of guidance and unclear expectations contribute to unstandardised ETS practice. Nurses perceived this as both unavoidable and a negative consequence "*I think it's almost every man for himself—just about—of how you interpret how it's done to some degree"* (S10) and "*techniques are quite variable in the unit … there's obviously a risk'* (S12). Nurses commented on the nature of PICU nursing and the difficultly standardising practice "*I don't think that there is any rule or protocol or policy that covers every patient that we have here in ICU" (S05)* and "*it's hard to standardise something because obviously every patient is different" (S09).*

## 4. Discussion

Study participants perceived nurses to be the key decision-makers in ETS care and reported integrating multiple sources of evidence to inform ETS practice decisions in the PICU. Nurses' uncertainty regarding the physiological effects of NSI and LR and their uncertainty when applying these interventions, in particular LR, was a key finding of this study which has not previously been reported in this context.

In the last decade, substantial focus has been on the evidence-based practice revolution and the translation of research evidence to guide clinical decisions. The evidence-based practice model recognises the value of research evidence to inform nursing practice decisions.[16] However, in PICU, few clinical interventions including NSI[10] and LR[33] have sufficient clinical trial data to inform safety and efficacy questions and best practice recommendations.[19] This is evident when considering the main indication nurses cited for NSI, "*thick secretions*". Study participants perceived dry ETS (no NSI) as having limited efficacy in terms of secretion mobilisation, particularly in children with tenacious secretions, a finding that aligns with other PICU ETS studies.[34] However, a well cited, benchtop study from the 70s showed normal saline and airway mucous to be immiscible,[35] raising the question why do nurses perceive an increased secretion clearance with NSI, that extends beyond the secretion volume?

Research undertaken in the 1980/90s found NSI had a positive effect on secretion volume and weight[36,37]; however, the authors describe this increase as not clinically meaningful. More recently, research undertaken in porcine airway mucosa found that aqueous salt solutions such as normal saline could hydrate airway surfaces and restore mucociliary transport.[38] These findings suggests NSI achieves its effect not through the dilution of respiratory secretions as previously proposed[35] but through the hydration of airway mucosa and subsequent restoration of mucociliary transport. These results, while tested in animal models and yet to be confirmed in human studies, offer a plausible explanation for nurses' perceptions of enhanced secretion clearance in ventilated children with thick airway mucous.

The safety of NSI is strongly debated in current literature. Lung mechanics markedly deteriorate after ETS due to pulmonary derecruitment,[12] often irrespective of NSI administration.[39] Whilst the association between NSI and oxygen desaturation is not definitively proven, studies which report significant difference in oxygen saturation describe a transient event with no studies reporting ongoing hypoxia after the 10 min mark.[10] In paediatric studies, limited conclusions and recommendations can be drawn from existing empirical studies which are limited by both size (one randomised controlled trial, $n = 24$[40]) and methodology (lack of random allocation).[34] Nurses in our study did not perceive negative clinical effects to be associated with NSI administration. This finding highlights the need for further observational work into the prevalence of ETS-related complications in the general PICU cohort. Future studies could measure the number of desaturation events which required clinician intervention (e.g. in rescue oxygenation) to provide a clinical indication of the perceived significance of the desaturation.

In our study, nurses perceived the greatest lack of evidence and guidance situated around the application of LR. Nurses were unsure of the correct technique (PEEP manipulation versus sustained inflation) and discussed perceptions of serious complications as a result of incorrect technique (e.g air leaks). Research undertaken in 60 mechanically ventilated children, found doubling the PEEP for two minutes after ETS significantly improved end-expiratory lung volume in children with "healthy lungs" and acute lung injury.[41] Conversely, when examining dynamic compliance, Morrow et al.'s[42] RCT of 34 mechanically ventilated children (48 recruited, 14 excluded from analyses) found that a sustained inflation (30 cm $H_2O$ for 30 s on manual bag) following ETS did not lead to a significant change in dynamic compliance in non-paralysed, ventilated, and spontaneous breathing children. Whilst this finding is limited by sample size, they suggest that LR may not improve lung mechanics following ETS as previously thought. Furthermore, whilst recruitment manoeuvres have been suggested as a means to regain lung volume after ETS, practical and clinical guidelines for the bedside clinician are lacking because of lack of safety and efficacy evidence. Consequently, PICU nurses are making LR decisions in a vacuum of evidence, and many study participants reported a lack of confidence to apply LR following ETS.

Overall, we found that whilst nurses perceived research findings as a valuable source of evidence, they perceived it to be difficult to access and translate to everyday practice. In the absence of ETS best practice recommendations, nurses expressed a preference for using clinical experience as their main source of evidence. Almost every participant referred to the importance of having previous clinical experience with a similar patient "*so you know what to expect in terms of disease progression and suction needs"*. Clinical experience as a source of information is widely recognised as an essential but fallible source of evidence.[43] Experienced nurses in the study reported considering individual patient characteristics such as medical diagnosis, disease progression, and thickness of secretions when determining ETS interventions. Conversely, less experienced nurses reported routine NSI use and an abstinence of LR application. These nurses discussed a perceived lack of experience and pathophysiologic reasoning (mechanism of action of NSI or LR) as the primary reasons for routine NSI use and failure to apply LR. While randomised controlled trials are appropriate methods for evaluating the efficacy and safety of complex interventions,[17,44] other sources of evidence including clinical experience are essential in PICU nurses' daily practice. No single source of evidence was sufficient to guide PICU nurses ETS decisions.

Variability in NSI and LR practice may be acceptable when based on the consideration of clinical guidelines, medical knowledge, or patient status.[44] However, integrating multiple sources of information and evidence is a process which can lead to practice variability,[45] which was evident in our findings. Clinicians need support to determine whether the application of guidelines is appropriate or an alternate approach should be employed. Extensive work is being undertaken by Australian researchers to develop and validate an Endotracheal Suction Assessment Tool, a decision making tool for assessing the clinical indication to perform an ETS.[28,46] Once validated, this tool will be particularly valuable to novice PICU nurses with limited experience and pathophysiological understanding of indications for ETS. However, further testing of NSI and LR is urgently needed to determine what safe and efficacious practice is. We do not know if current ETS practice in the context of NSI and LR is ultimately causing harm as has been suggested in the case of NSI. Rigorous clinical trial data will enable the translation of these findings into clinical guidelines and best practice recommendations, thereby constructing knowledge to support all levels of PICU nurses. Further, we need to support nurses to understand the physiological mechanisms of ETS and related interventions as this is an important first step in nurses' decision-making.

In this study, we report useful insights into PICU nurses' experiences with NSI and LR; however, there are some limitations. We drew from a single site where open ETS technique was predominantly used. Although we achieved saturation of data, the resulting themes may not be generalisable to units using closed ETS where alternative treatment considerations may have ensued. Our findings therefore provide an initial understanding of the influencing factors nurses apply to NSI and LR interventions in the PICU; yet, further research is needed to determine what is ETS "best practice" in this vulnerable and heterogeneous population.

## 5. Conclusion

Variability in nurses' NSI and LR practice in ETS was marked. In the absence of evidence-based clinical guidelines, nurses relied on knowledge of individual patient's condition, clinical knowledge and experience, and the local setting expectations to guide NSI and LR intervention decisions. The development of ETS protocols without the consideration of the multiple sources of evidence may be problematic and result in poor clinician uptake or adherence. The generation of paediatric ETS best practice guidelines is urgently needed; however, this should occur in consultation with key stakeholders including bedside clinicians.

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## Authors' contributions

JS conceived the study, developed the protocol, wrote funding applications, completed data collection and analysis, and drafted and revised the final manuscript. MC and MM conceived the study, developed the protocol, assisted with data analysis and revised, and approved the final manuscript. DL assisted with protocol development and revised and approved the final manuscript.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.aucc.2018.03.002.

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