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The correlation between in-situ duration of neonatal feeding tubes and the bacterial colonisation

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Abstract

Neonates with incompetent immune system in the neonatal intensive care unit (NICU) are at risk of developing infection from microbial colonisation of enteral feeding tubes. The study aimed to identify the bacteria present in the enteral feeding tubes of neonates admitted to the NICU of the Canselor Tuanku Muhriz Hospital Malaysia. The feeding tubes were assessed after being placed in the stomach for 24, 48 and 72 hrs for each neonate. A total of thirty tubes from ten neonates were tested for enumeration of aerobic colony count, *Staphylococcus* aureus, Enterobacteriaceae, *Enterococcus* spp. and presence of *Cronobacter sakazakii* and *Salmonella* spp. The duration of feeding tubes in-situ was not significantly correlated with aerobic colony count, *S. aureus*, Enterobacteriaceae and *Enterococcus* spp. colonisation with Spearman's rank correlation coefficient r_s of +0.116, -0.146, -0.115, and +0.287 respectively. *Klebsiella pneumoniae*, *Citrobacter freundii*, *Yersinia enterocolitica, Enterococcus faecium, E. malodoratus, Streptococcus anginosus* and *Pediococcus pentosocaeus* were found in the tested samples and their presence was considered as risk of infection as most being part of Enterobacteriaceae. *Cronobacter sakazakii* and *Salmonella* spp. was not detected in any of the tubes. In conclusion, bacterial colonisation in feeding tubes was not associated with duration in-situ for 24, 48 and 72 hrs.

1. Introduction

Neonatal Intensive Care Unit (NICU) admits premature and other neonates who require intensive care. These neonates are at risk of infection due to several factors including immaturity of the immune system (McKenney, 2001). Centers for Disease Control and Prevention (CDC) reported that the infection outbreaks in NICU are commonly caused by several organisms including *Acinetobacter,* Enterobacteriaceae, Vancomycin-resistant Enterococci (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Centre of Disease Control and Prevention (CDC), 2014).

Premature neonates have poor sucking reflexes and are usually given milk including formula feeding via oral gastric (OG) tubes for up to several weeks (Boo, 2016). Neonatal feeding tubes consist of oral gastric and nasogastric which are being practised in NICU (Liu *et al.,* 2015). These tubes are inserted via the mouth and the tips are placed in the stomach or upper small intestines of the neonates (Kim, 2011). Feeding tubes are made of polyvinylchloride (PVC), polyurethane or silicone (Wallace and Steward, 2014) and may act as a site for bacterial colonisation since the bacteria has the ability to form biofilm on feeding tubes. Biofilm on the feeding tubes may detach and pass into the intestine, consequently exposing the neonates to the risk of infection (Kim *et al.,* 2006).

The bacteria may originate either from unpasteurised milk, the infant's gut or contamination of parents' and milk handlers' hands during milk handling (Hurrell *et al.*, 2009; Mehall *et al*., 2002). The presence of residual milk in feeding tubes, the ambient temperature of the feeding tubes and the regular supply of nutrients, provide an ideal condition for bacterial colonisation. Bacteria were found in various feeding regimes such as breast milk, fortified breast milk, ready-to-feed formula, reconstituted milk and mixed feeding (Hurrell *et al.,* 2009). It is reported that microbial colonisation in

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neonatal feeding tubes is by *Candida*, *Staphylococcus* spp. and Enterobacteriaceae such as *Enterobacter cancerogenus, Serratia marcescens, E. hormaechei, E. coli* and *K. pneumoniae*. Some isolates are also antibiotic resistant towards ceftazidine, cefotaxime, amoxicillin and co-amoxiclav. (Juma and Forsythe, 2015). It is supported by Jara Pérez *et al*. (2021) that bacterial colonisation in feeding tubes can cause antibiotic resistant. Bacterial communities inside feeding tubes could act as reservoirs of antibiotic resistance genes. Recent review study by Parker *et al.* (2022) found that neonatal feeding tube contains pathogenic bacteria, antibiotic resistant bacteria and longer placement increased the bacterial counts. Mehall *et al*. (2002) found that 71 from 125 feeding tube samples in tertiary children's hospital in the United States were contaminated with more than 10^3 CFU/mL of different types of organisms such as *S. aureus*, *Enterococcus faecalis, E. cloacae* and *K. pneumonia*. Hurrell *et al.* (2009) and Kim *et al.* (2006) reported that the Enterobacteriaceae colonisation in feeding tubes was not influenced by the types of feeding regimes. Neonates in this NICU received different types of feedings such as ready-to-feed milk (RTF), pasteurised expressed breast milk (PEBM), freshly expressed breast milk (FEBM), powdered infant formula milk (PIF), fortified feedings and mixed feedings. The mixed feeding of breast milk, fortified breast milk and PIF showed significantly higher counts than 'nil by mouth' except for the ready to feed formula and breast milk feeding (Hurrell *et al.*, 2009). The 'nil by mouth' means nothing through the mouth and was determined as a control group.

Alkeskas *et al.* (2015) found *Escherichia coli* strains isolated from residual liquids. *Escherichia coli* K1 was isolated in that study and was known as virulence traits associated with 80% neonatal meningitis. A study by Gómez *et al.* (2016) reported that biofilm can be found in internal feeding tubes, external feeding tubes, and connectors. The bacterial flora of nasogastric feeding tubes and faecal samples were analysed for a low-birth weight (725 g) neonate with estimated gestational age of 25 weeks in intensive care. The bacterial biofilm and faecal samples included *E. faecalis* and *E. hormaechei* (Ogrodzki *et al*., 2017). Neonatal feeding tube contains pathogenic bacteria, and longer placement increased the bacterial counts (Parker *et al.*, 2022). An observational study by Petersen *et al*. (2016) revealed that there was no correlation between the in-situ duration of feeding tubes for one day insertion to microbial colonisation. However, it was found that higher contamination of feeding tubes left in-situ for four days (mean log 6.9 CFU/g) (Abdullah Sani *et al.,* 2012) compared to three days (mean log 4.7 CFU/g) (Abdullah Sani and Loo, 2013) in the NICU of Malaysian hospitals. Bacteria also can grow up to 10^7 CFU/mL within 8 h and reached 10^9 CFU/mL in 24 hrs in feeding tubes (Hurrell, Kucerova, Loughlin, Caubilla-Barro. and Forsythe, 2009). These findings suggested that duration of feeding tubes in-situ may be associated with the number of bacterial multiplication and colonisation. The common practice of changing the feeding tubes in the NICU of this study was that the feeding tubes were changed after three days. Therefore, this study was conducted to evaluate the bacterial colonisation within three days with time interval after day one (24 hrs), day two (48 hrs) and day three (72 hrs). The correlation between the duration of feeding tubes in-situ and bacterial colonisation of the tubes for 24 hrs, 48 hrs and 72 hrs were determined.

2. Materials and methods

2.1 Samples collection and preparation

This study was conducted at the NICU of the Canselor Tuanku Muhriz Hospital (HCTM), Kuala Lumpur, Malaysia. The study protocol was approved by the Research and Ethics Committee of the Universiti Kebangsaan Malaysia (UKM1.5.3.5/244/FST-2015-013). The inclusion criterion was neonates who were on enteral feeding via feeding tubes. Written informed consent was obtained from the parents prior to enrolment into the study. Feeding tubes were inserted via the mouth (oral gastric) or nose (nasogastric) by the attending nurses according to standard procedure. The feeding tubes in this study were made of polyvinylchloride (PVC). Feeds were administered by the nurses every two to three hours by pouring into a sterile syringe (without plunger) attached to the feeding tube. Feeds were consisted of pasteurised expressed breast milk (PEBM), fresh expressed breast milk (FEBM), ready to feed milk (RTF), powdered infant formula (PIF) with and without human milk fortifier (HMF), carborie, medium-chain triglyceride (MCT) oil and myotein.

Feeding tubes were removed after 24 hrs, 48 hrs and 72 hrs being in-situ for each of the neonates (A-J). Three time periods were used to determine the bacterial colonisation in less than 72 hrs because the current practice is placing the tube for up to 3 days. This is to ensure that it is safe to leave the tubes in-situ for up to 72 hours duration without removing them at 24 or 48 hours, from bacterial contamination.

Upon removal, the enteral feeding tubes were kept in 3M™ sterile bag labelled with details (patients' registration number and date of insertion and removal) and stored in the freezer (-18°C). The tubes were then transported in a cool box (temperature maintained at < 5° C) to the Food Microbiology Research laboratory in the Faculty of Science and Technology, UKM (Bangi

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campus, Selangor, Malaysia) for analysis.

2.2 Microbiological analysis

The outer surface of each feeding tube sample was swabbed with 70% ethanol (Merck, Germany) and aseptically cut into a few pieces. A total of 1.0 g tubes were homogenised with 9 mL of maximum recovery diluent (MRD, Oxoid) resulting in a 1:10 homogenate and serial dilutions were performed until five serial decimal dilutions (1:100, 1:1000, 1:10000 and 1:100000). The enumeration was done by plating on 3M™ Petrifilm™ Aerobic Colony Count (AOAC 990.12, 2002), 3M™ Petrifilm™ Enterobacteriaceae (AOAC 2003.01, 2003), Rabbit plasma fibrinogen agar for *S. aureus* (Oxoid) (ISO 6888-2:1999) and KF *Streptococcus* agar for *Enterococcus* (Oxoid) (BS4285 Sec 3.11). Typical Enterobacteriaceae and *Enterococcus* spp. were confirmed using RapID™ ONE (Remel™), and RapID™ STR (Remel™) respectively.

For the detection of *Salmonella* (ISO 6579:2002) and *Cronobacter sakazakii* (ISO 22964:2006), a total of 0.5 g tube was vortexed with buffered peptone water (BPW, Oxoid) and incubated at 37°C for 18-24 hrs for pre-enrichmE. The samples were then enriched using Rappaport-Vassiliadis Enrichment broth (RVS, Oxoid) and Muller-Kauffmann Tetrathionate Novobiocin broth (MKTTN, Oxoid) and streaked on surface of Xylose Lysine Desoxycholate agar (XLD, Oxoid) and Salmonella Shigella agar (SSA, Oxoid) for detection of *Salmonella*. As for the detection of *C. sakazakii*, *Cronobacter* Screening broth with Vancomycin (CSB, Oxoid) was used for enrichment at 42°C and the enriched samples were then streaked on Chromocult® *Enterobacter sakazakii* agar (CES, Merck) and incubated at 37°C for 24 hrs. This research was conducted in January to April 2016.

Data were analysed using SPSS 25.0 (SPSS, Inc., Chicago, IL, USA). A statistical comparison between bacterial colonisation of feeding tubes and duration in-situ was tested using One-way ANOVA with p value of < 0.05 taken as statistical significance. The Spearman's correlation coefficient was used to determine the correlation between in-situ duration and bacterial colonisation of feeding tubes.

2.3 Scanning of biofilm formation

The presence of biofilm on the interior surface of feeding tubes was visualised by Scanning Electron Microscopy Zeiss Gemini SUPRA 55-VP, (Zeiss, Germany) following the method of Hurrell, Kucerova, Loughlin, Caubilla-Barro. and Forsythe (2009). Feeding tubes were cut into 1 cm pieces and fixed in 2% glutaraldehyde for 12 to 24 hrs at 4°C. Samples were rinsed with 0.1 M phosphate buffer saline (Oxoid) for 10 mins three times. Samples were dehydrated with 30%, 50% 70%, 80%, and 90% ethanol (Merck) for 10 min and 100% ethanol for 15 min. Samples were dried on Leica EM CPD 300 Critical Point Dryer (Leica, Austria) for 30 min and then were subsequently mounted with platinum (Bio-Rad SC 500, (Bio Rad United States). The inner surface of the feeding tubes was examined with a FESEM Zeiss Gemini Supra 55 VP (Zeiss, Germany) to visualise the presence of biofilm.

3. Results and discussion

3.1 Bacterial colonisation

A total of thirty enteral feeding tubes from ten neonates were tested in this study. The neonates had a mean (SD) gestational age of 32.3 (0.67) weeks and their birth weight ranged between 1.0 to 2.5 kg. The neonates received different types of feeding: 46.7% were fed with PEBM with HMF, 13.3% were fed with PEBM, 10.0% were fed with mixture of RTF and PEBM with HMF and MCT oil, 6.7% were fed with mixture of RTF and PEBM with PIF, myotein and MCT oil, and 3.3% were fed with a mixture of RTF, fortified PEBM and FEBM with HMF and Carborie. Bacterial parameters and colonisation in feeding tubes for 24, 48 and 72 hrs as shown in Table 1 and Table 2 respectively. Neonates in this NICU received different type of feedings such as RTF, PEBM, FEBM, PIF, fortified feedings and mixed feedings. The mixed feeding of breast milk, fortified breast milk and PIF showed significantly higher counts than 'nil by mouth' except for the RTF and breast milk feeding (Hurrell *et al.*, 2009). The 'nil by mouth' means nothing through the mouth and was determined as a control group. According to Mehall *et al.* (2002), feeding tube was classified as "contaminated" with total bacteria $>10^5$ CFU. Parenteral and Enteral Nutrition Group of British Dietetic Association stated that the acceptable limit for aerobic count in the feeding tube is 10^1 at the beginning and 10^3 during feeding. Therefore, Feeding tube containing more than 10^3 CFU was not considered safe (Anderton, 1993). Based on the result, 23 (76.7%) feeding tubes were not safe as contaminated with aerobic count $>10^3$ CFU. Aerobic colony count was found in 93.3% of tube samples with the highest count recorded at 72 hrs (mean log 4.37 CFU/g). The highest aerobic count among 30 tube samples was in the feeding tube of neonate D (48 hrs) fed with mixed feeding of RTF and fortified PEBM with HMF (7.15 mean log CFU/g). HMF is commonly added into EBM for newborns (born < 32 weeks and bodyweight < 1.5 kg) (Hussain Iman *et al.,* 2019). HMF in milk can increase body weight and growth. HMF used in this NICU is in powdered form (sachet).

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Table 1. Bacterial parameters in feeding tubes for 24, 48 and 72 hrs placement periods

Parameter	Duration of feeding tubes placement (hrs) / Bacterial colonisation (mean log CFU/g)			P value
	24 hrs	48 hrs	72 hrs	
Aerobic colony count	3.43	4.2	4.37	0.49
Staphylococcus aureus	1.48	1.21	0.88	0.68
Enterobacteriaceae	0.7	0.78	0.25	0.37
Enterococcus spp.	0.77	1.17	1.76	0.39
Cronobacter sakazakii	ND	ND	ND	
Salmonella spp.	ND	ND	ND	

ND: Not detected

Table 2. Bacterial colonisation in feeding tubes for 24, 48 and 72 hrs placement period

Neonate	Tube placement period (hrs)	Feeding	Aerobic colony count	*S. aureus*	Enterobacteriaceae	*Enterococcus* spp.	*C. sakazakii* and *Salmonella* spp. (CFU/g)
			Mean log CFU/g				
A	24	PEBM+HMF	2.85^{b}	0.00^{a}	0.00^{c}	0.00^{a}	ND
	48	PEBM	3.68^{a}	0.00^{a}	1.88^{a}	0.00^{a}	ND
	72	PEBM +HMF	2.85^{b}	0.00^{a}	1.18^{b}	0.00^{a}	ND
B	24	PEBM+HMF	5.18^{a}	2.82^{a}	1.30^{a}	0.00^{b}	ND
	48	PEBM+HMF	3.38^{b}	0.00^{b}	0.30^{b}	0.00^{b}	ND
	72	PEBM+HMF	4.84^{c}	0.00^{b}	0.00^{c}	2.26^{a}	ND
C	24	PEBM+HMF	0.00^{c}	0.00^{a}	0.00^{a}	0.00^{a}	ND
	48	PEBM+HMF	1.66^{a}	0.00^{a}	0.00^{a}	0.00^{a}	ND
	72	PEBM+HMF	1.60^{b}	0.00^{a}	0.00^{a}	0.00^{a}	ND
D	24	PEBM+HMF	6.57^{b}	4.07^{a}	1.30^{a}	0.00^{b}	ND
	48	RTF, PEBM+HMF	7.15^{a}	4.08^{a}	0.00^{b}	0.00^{b}	ND
	72	PEBM+HMF	7.14^{a}	2.00^{b}	0.00^{b}	3.93^{a}	ND
E	24	RTF+PEBM	0.00^{c}	0.00^{a}	0.00^{b}	0.00^{c}	ND
	48	RTF+PEBM	6.11^{a}	0.00^{a}	0.00^{b}	4.41^{a}	ND
	72	FEBM+HMF+ CARBORIE	4.70^{b}	0.00^{a}	1.30^{a}	3.40^{b}	ND
F	24	PEBM+HMF	4.40^{c}	0.00^{a}	0.00^{b}	0.00^{b}	ND
	48	PEBM+HMF	5.43^{b}	0.00^{a}	2.04^{a}	0.00^{b}	ND
	72	PEBM+HMF	5.95^{a}	0.00^{a}	0.00^{b}	2.78^{a}	ND
G	24	RTF	1.70^{c}	0.00^{b}	0.00^{a}	1.40^{a}	ND
	48	RTF	3.00^{b}	1.80^{a}	0.00^{a}	1.18^{b}	ND
	72	PEBM+HMF	3.79^{a}	0.00^{b}	0.00^{a}	1.00^{c}	ND
H	24	PIF, MYOTEIN, PEBM, MCT OIL	5.04^{a}	2.46^{b}	0.00^{b}	2.11^{a}	ND
	48	PIF, MYOTEIN, PEBM, MCT OIL	4.85^{b}	0.00^{c}	2.92^{a}	1.91^{b}	ND
	72	RTF	4.67^{c}	2.62^{a}	0.00^{b}	0.00^{c}	ND
I	24	PEBM	4.76^{bc}	1.81^{abc}	2.18^{a}	1.15^{a}	ND
	48	PEBM	5.18^{bc}	3.00^{abc}	1.00^{a}	1.15^{a}	ND
	72	PEBM	4.15^{bc}	2.18^{abc}	0.00^{a}	1.18^{a}	ND
J	24	PEBM+HMF+ MCT OIL	3.76^{c}	3.63^{a}	0.00^{a}	0.00^{a}	ND
	48	PEBM+HMF+ MCT OIL	4.26^{a}	2.56^{b}	0.00^{a}	0.00^{a}	ND
	72	PEBM+HMF+ MCT OIL	4.04^{b}	2.40^{c}	0.00^{a}	0.00^{a}	ND

Values with different superscripts within each row are statistically significant different.

FEBM: fresh expressed breast milk, PEBM: pasteurised expressed breast milk, RTF: ready to feed milk, HMF: human milk fortifier, PIF: powdered infant formula, MCT: medium-chain triglyceride, ND: not detected

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As indicated by Jocson *et al.* (1997), human milk fortifiers can provide an ideal condition for bacterial growth in breast milk. This could be a reason for the bacterial colonisation in feeding tubes of neonates fed with fortified breast milk. HMF also has been reported to be contaminated with *C. sakazakii* and *Salmonella* (Ministry of Health Malaysia, 2022). However, no *C. sakazakii* and *Salmonella* were found in all feeding tube samples of this study. HMF and protein powder (Myotein) were common nutritional supplements added in breast milk in this NICU. Fortifiers were added into fresh or pasteurised EBM after warming with automated dry warmer (Beldico, Belgium) at 37°C for 30 mins prior to feeding. The fortification process was done in milk preparation room and then brought to the bed area to avoid cross-contamination from the ward environment. However, contamination of fortified milk feeding may occur due to mishandling.

The highest rate of contamination for *S. aureus* in this study was found in feeding tubes at 24 hrs with mean log 1.48 CFU/g. Mehall *et al.* (2002) isolated three different types of bacteria including *S. aureus* with a mean log 5.96 CFU/g in 71 neonatal tube feedings in-situ for one week. *S. aureus* were isolated in 13 feeding tubes (43.3%) in this study at different in-situ duration (24 hrs = 16.7%, 48 hrs = 13.3%, 72 hrs = 13.3%). Petersen *et al.* (2016) also found 13 feeding tubes (13.8%) contaminated with $>10^3$ CFU/mL of *S. aureus*. Contamination of feeding tubes with *S. aureus* is well-associated with improper handling of milk (Borges *et al*., 2010). It can be contaminated by the hands of milk technicians during milk preparation, the hands of nurses during feed administration or mothers' hands during milk pumping. Recent study found *S. aureus* (55.7%) in contaminated expressed breast milk samples (Gad *et al.*, 2021). In this NICU, milk preparation and handling followed a strict protocol and supervised by the head nurses. Mothers were monitored by nurses on hygiene practices during pumping of breast milk in the NICU. Mothers were also advised on hygienic practices during pumping at home or in the ward by the nurses. The presence of *S. aureus* in a few samples of tube feedings may be derived from the contaminated hands during milk fortification. Handling milk aseptically can reduce contamination of PEBM during fortification with HMF (Fenton and Belik 2002).

The highest Enterobacteriaceae were isolated from feeding tubes in-situ at 48 hrs (mean log 2.92 CFU/g) Mehall *et al.* (2002) reported that out of 71 contaminated feeding tubes, only 10 feeding tubes were contaminated with Enterobacteriaceae at $>10^5$ CFU/g, higher than this study. *Klebsiella pneumoniae, C. freundii* and *Y. enterocolitic*a were found in contaminated neonatal feeding tubes in this study. *Klebsiella pneumoniae* were isolated from feeding tubes of neonate A (48 hrs and 72 hrs) and neonate I (48 hrs). *Citrobacter freundii* was isolated from neonate B (24 hrs), neonate D (24 hrs), neonate H (48 hrs) and neonate I (24 hrs). *Yersinia enterocolitic*a found in neonate B (48 hrs) and neonate E (72 hrs). The presence of *K. pneumoniae, C. freundii* and *Y. enterocolitic*a in the feeding tubes provide high risk to the neonates. *Klebsiella pneumoniae* and *C. freundii* found in the tubes may be derived from the contaminated milk feedings. Dorota *et al.* (2017) found *K. pneumoniae* in mothers' milk which can cause sepsis. Based on the NICU sampling report in 2017 to 2018, *K. pneumoniae* was isolated from mother's milk expressed at home and in the NICU, while *Citrobacter* spp. was also reported present in PIF and follow-up formulas (Abdullah Sani *et al.,* 2013). Hurrell *et al.* (2009) reported that *Y. enterocolitica* and *C. freundii* were found in feeding tubes (< 6 hrs to 48 hrs in-situ). In this NICU, fresh EBM were kept refrigerated (<4°C) up to 48 hrs prior to feeding. Chilling fresh EBM under suitable temperature can minimise bacterial growth.

The highest count of *Enterococcus* spp. was found in feeding tubes of 72 hrs in-situ with mean log 1.76 CFU/g. *Enterococcus faecium, S. anginosus, P. pentosaceus* and *E. malodoratus* were found in contaminated neonatal feeding tubes. It is supported by Filleron et al. (2013) who reported that *Streptococcus* Group B was found in pasteurised breast milk. *Streptococcus anginosus* can cause intracranial complications of rhinosinusitis among paediatrics (Deutschmann *et al*., 2013). Petersen *et al.* (2016) found 25 feeding tubes (26.6%) contaminated with *Enterococcus* spp. The presence of *E. faecium* in neonatal feeding tubes was reported in NICU ward in a previous study (Dahmani, 2018). Thus, a heat treatment process (pasteurisation at 62.5°C for 30 mins) is needed to eliminate *Enterococcus* spp. in breast milk (Lima *et al.*, 2017). Pasteurisation is a heat treatment process that can eliminate bacteria or virus that may be present in human milk (Robbins and Meyers, 2011). Breast milk bacteria were primarily derived from areolar skin and infant which were comprised of *Staphylococcus, Streptococcus, Acinetobacter*, and *Enterobacter* (Kordy *et al.,* 2020). Holder pasteurisation did not kill all bacteria but reduced bacteria such as Staphylococci coagulase-negative, Gram-negative bacteria such as *Enterococcus* spp. (Klotz *et al*., 2017; Wesolowska *et al.,* 2019).

Based on this NICU routine practice, all EBM which was expressed at home must be pasteurised in the milk room. The EBM were kept in a designated freezer with temperature of -18°C or less, for up to 3 months. The frozen EBM were thawed and then pasteurised using an

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automated pasteuriser (Sterifeed S180, United Kingdom) at 62.5°C for 30 mins to eliminate the presence of bacteria in EBM. One of the feeding tubes of a neonate fed with fresh EBM (un-pasteurised EBM) in this study was contaminated with the *Enterococcus* spp. indicating that the pasteurisation process was needed to eliminate *Enterococcus* spp. in EBM. The high contamination rate of *Enterococcus* spp. in feeding tubes was also found related to faecal-oral transmission (Mehall *et al.,* 2002). *Enterococcus* spp. is commonly found in faeces and it can easily be transmitted due to poor hygiene practices such as improper washing hands after using the toilet. Noskin *et al.* (1995) also noticed the survival of *E. faecalis and E. faecium* on gloves and fingertips without gloves for 60 min. This can lead to the presence of *Enterococcus* spp. in feeding tubes caused by contaminated hands.

Cronobacter sakazakii and *Salmonella* spp. were not detected in any feeding tubes removed after 24, 48 and 72 hrs from the neonates. *Cronobacter sakazakii* and *Salmonella* spp. are commonly found in contaminated PIF. The non-presence of these species in this study was because the milk handlers in this NICU reconstituted PIF with boiled water > 70°C as suggested by FAO/WHO (2007) to reduce pathogens in PIF. However, Hurrell *et al.* (2009) in their study revealed that *C. sakazakii* was also isolated from the enteral feeding tubes of two neonates receiving breast milk and RTF formula. The RTF formula remains sterile until it is opened. In this NICU, RTF bottles were usually opened just before use and the excess RTF formula was immediately discarded. Aseptic techniques should also be applied to prevent cross contamination *C. sakazakii* and *Salmonella* spp. are known to be well-associated with contaminated PIF (Food and Agriculture Organization/World Health Organization, 2006; Abdullah Sani *et al*., 2014). About 12.5% prevalence of *C. sakazakii* contamination from 72 PIF samples in Malaysia was reported in a previous study (Abdullah Sani *et al.,* 2014). It is supported by Yusof *et al*. (2017) who found an increase in *Cronobacter* spp. biofilm formation with the presence of milk. RTF formula is commercialised sterile milk and stored at room temperature in the milk preparation room. The risk of RTF formula contamination may occur after the bottle is opened. According to BDA (2016), RTF is free from microbiological contamination before it is opened. In this NICU, RTF formula bottles were usually opened just before use and the excess RTF formula was immediately discarded. The aseptic technique was applied to prevent cross-contamination.

Based on this study, there was no significant correlation between the duration in-situ of feeding tubes (24, 48 and 72 hrs) and its colonisation of feeding tubes with aerobic organisms, *S. aureus*, Enterobacteriaceae and *Enterococcus* spp. (Spearman's rank correlation coefficient, r_s = +0.116, -0.146, -0.115, +0.287 respectively). Petersen *et al.* (2016) also reported that all tested bacterial colonisation was not correlated with duration in situ of feeding tubes (r_s = 0.065, p = 0.53) but showed a weak positive correlation between the age of infants at tube collection and the level of bacterial contamination (r_s = 0.15, P = 0.16). It shows that the bacterial colonisation in neonatal feeding tubes in this study was not influenced by the length of time the feeding tube was used.

3.2 Biofilm formation

Figure 1 shows the presence of bacterial biofilm on the interior feeding tubes of neonate E for 24, 48 and 72 hrs in situ. Rod-shaped bacterial biofilm found may be Enterobacteriaceae that had been isolated from feeding tubes of neonate E (72 hrs in situ) via microbiological analyses. Coccus-shaped biofilm bacteria found in feeding tubes of neonate E (24, 48 and 72 hrs) in situ may have derived from *S. aureus* and *Enterococcus* spp. that had been isolated. Coccus-shaped bacteria found in feeding tubes of neonates C and E (24 hrs) were not *S. aureus* or *Enterococcus* spp. It may have derived from other species such as *Streptococcus* spp. that can be present in contaminated breast milk (Burianova *et al.,* 2013). It is supported by Filleron *et al*. (2013) who reported that *Streptococcus* Group B was found in pasteurised breast milk as neonates C and E were also fed with pasteurised breast milk.

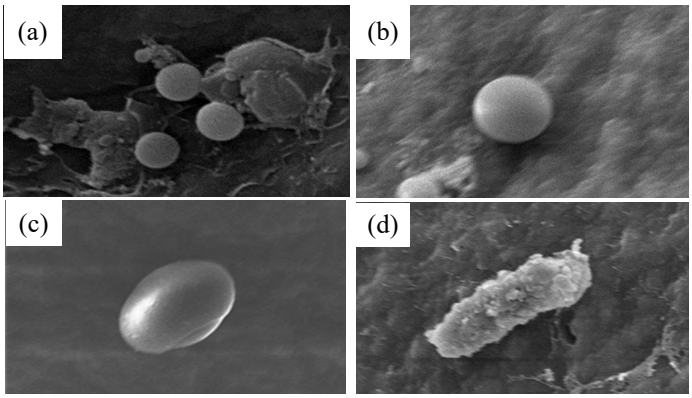

Figure 1. Scanning electron microscopy images of the inner surface feeding tube of neonate E (a) 24 hrs placement (b) 48 hrs placement and (c, d) 72 hrs placement. Magnifications: (a) 1000×, (b) 300×, (c) 200×, (d) 200×

Meanwhile, bacterial colonisation in the intestinal tract was reported to be influenced by the bacterial flora in neonatal feeding tubes (Ogrodzki *et al.,* 2017). A recent finding from Taft *et al.* (2019) reported that bacterial colonisation in neonatal feeding tubes is similar to bacterial found in faecal samples of infants fed with breast milk compared to infant formula milk. The risk of

https://doi.org/10.26656/fr.2017.7(3).001

bacterial colonisation in the feeding tubes can be reduced by improving the feeding administration and preparation practices in the NICU.

Feeding administered to neonates and infants via feeding tubes should be handled aseptically. Standard operating procedure (SOP) for preparing milk to feed infants via feeding tubes is being practised in the NICU. Nurses wore proper attire with sterile gloves and aprons during the administration of feeds via feeding tubes. A flushing technique with air-flush using an empty syringe was conducted prior to and after feeding to remove the excess milk in the tube. The excess milk can be a potential risk of neonatal infection and it could be reduced by improving the feeding practise in this NICU. However, there are no evidence that flushing with sterile water can reduce contamination in feeding tubes.

Other researchers have reported that the implementation of the Hazard Analysis and Critical Control Points (HACCP) system can reduce the bacterial contamination of feeds in enteral feeding (Oliveira *et al.,* 2001; Cossey *et al.,* 2011). Implementation of the HACCP system will change the staff's routine practices in milk preparation and consequently improve the microbiological quality of enteral feeds (Oliveira *et al.,* 2001). Hazard analysis (Principle 1 HACCP) will help to identify the potential hazards and risks in milk preparation in NICU. HACCP system also will identify the critical control point (CCP) that need to be monitored, so the contamination of enteral feeding tubes due to contamination of milk feeding can be reduced.

4. Conclusion

This study concludes that the duration in-situ was not correlated with bacterial colonisation in neonatal feeding tubes. Bacterial colonisation in the feeding tube was not influenced by the placement period (24 hrs, 48 hrs and 72 hrs) Further research on microbiological analysis of milk feeding samples inserted in feeding tubes should be tested to determine the source of contamination.

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