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CLINICAL RESEARCH

Appropriate handling and storage reduce the risk of bacterial growth in enteral feeding systems reused within 24 hours

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Abstract

Background: Enteral tube feeding can require considerable amounts of plastic equipment including delivery sets and containers, often disposed of after a single feeding session because of bacterial contamination concerns. The aim of this research was to assess whether reuse of delivery sets and containers for up to 24 h is safe from a microbiological perspective.

Methods: Four enteral tube feeding systems (FS) were tested under hygienic controlled or repeated inoculation challenge conditions using key foodborne pathogens, to assess bacterial growth over time (FS1: ready-to-hang, closed 1-L system with delivery set reused, stored at room temperature [RT]; FS2: a prepared, powdered, open 1-L system with delivery set and container reused, stored at RT; FS3 and FS4: prepared, powdered, open 200-ml bolus systems with delivery set and container reused, stored at RT [FS3] and refrigeration [FS4]). Feed samples were cultured at 0.5, 6.5, 12.5, 18.5, and 24.5 h with >2 Δlog considered significant bacterial growth.

Results: Under hygienic control, FS1, FS3, and FS4 were below the level of enumeration (<5 CFU/g) for all bacteria tested, at all time points. In FS2, significant bacterial growth was observed from 18.5 h. Under repeated bacterial inoculation challenge, no significant growth was observed in FS1 and FS4 over 24.5 h; however, significant growth was observed in FS2 after 6.5 h and in FS3 after 10–12 h.

Conclusion: With hygienic handling technique, there is limited bacterial growth with reuse of delivery sets and containers over 24 h. Refrigeration between feeding sessions and using boluses of reconstituted powdered feed reduce bacterial growth risk.

KEYWORDS
bacterial contamination, enteral nutrition, food safety, patient safety

INTRODUCTION

Enteral tube feeding is a valuable method of nutrition support to ensure safe and sufficient delivery of nutrition directly to the gastrointestinal tract. Frequently used in hospital and community settings, enteral tube feeding supports both acute and chronically ill patients when oral feeding is not possible or insufficient to meet nutrition requirements.[1] In some patients, enteral tube feeding may provide the sole source of nutrition; therefore, it is essential for life. In the United Kingdom, it is estimated that >23,000 adults receive community-based, long-term home enteral tube feeding (HETF),[2–4] and HETF incidence across Europe has been estimated to be between 62 and 457 new patients per million inhabitants per year.[5–7] Historically, enteral tube feeding has been considered a potential source of infection because of the risk of bacterial contamination of feed products, water, and administration equipment, with possible detrimental effects of administering contaminated feeds to patients including bacteriemia, septicemia, pneumonia, diarrhea, vomiting, and infectious enterocolitis.[8–13] However, after considerable research undertaken in the 1990s and early 2000s, improved feed sterilization techniques, improved design and sterility of tube feeding administration equipment[8,14–23] and the use of hygienic handling techniques by users,[24–28] have reduced the risk of bacterial contamination considerably.[29–37] Comprehensive guidance from healthcare professional bodies and enteral tube feed manufacturers on appropriate feed hanging times (typically 24 h for a sterile, ready-to-hang liquid feed and 4 h for a powdered, reconstituted feed, although this may vary between countries and manufacturers),[38,39] have also been implemented to reduce bacterial contamination risk. In addition, the relatively low cost to healthcare systems and the disposability of single-patient-use or single-use feeding administration equipment has likely further discouraged long-term use or reuse and potentially further reduced bacterial contamination risk. Faulty handling procedures during assembly and manipulation of the enteral tube feeding administration equipment have been cited as an important potential route for contamination,[10,14,15,17,27,39–41] which may occur during feed preparation (in the case of powdered/reconstituted feeds made up with water or mixing of multiple liquid feeds), or during manual handling of the equipment at "touch points" when connecting equipment together.[27] Tube feeding administration equipment typically used for feeding via a mechanical feeding pump consists of a delivery set (also known as a giving set), which attaches to the patients' feeding tube at the distal end and at the proximal end to either

- a commercially available, sterile, ready-to-hang plastic bottle or pouch, prefilled with a sterile liquid feed or
- an empty plastic container/reservoir into which a powdered feed mixed with water is added, or multiple liquid feeds are added.

Many delivery sets also possess a drug port, into which medications can be administered, which may provide a further contamination route. Other feed administration equipment may include enteral syringes, which can be used to manually administer water, medications or small "bolus" amounts of feed, directly into the patient's feeding tube. Pump feeding regimens may require administration of relatively small (bolus) volumes of feed (ie, 200 ml, known as "pump bolus feeding") delivered in short periods of time (ie, 30 min) on several occasions throughout the day,[42] or much larger volumes (ie, 1 L) delivered more slowly over a longer period of time, including sometimes continuously over 24 h.[42–46] To meet the needs of these various types of feeding regimens, feeding administration equipment has been designed either for use by a single patient in a 24-h period, or as single-use only (ie, used once and then disposed of). The differences in use and this terminology of "single-use only" have led to variations in clinical practice, and anecdotal evidence suggests that some patients may be prescribed up to six delivery sets and containers per day with new feeding administration equipment used for every bolus feeding session, or after every disruption in continuous feeding (ie, due to healthcare interventions, or activities of daily life). Whereas other patients who have the same feeding regimen maybe prescribed only one delivery set and container per day, and "reuse" the equipment throughout the day. Feeding regimens using up to six delivery sets and containers per day can lead to considerable cost to healthcare systems, a significant burden on storage space in healthcare and home settings, and considerable plastic waste or recycling needs. Therefore, exploration of practices to understand this variation in clinical practice and to reduce plastic waste in enteral feeding are required, to support governmental and healthcare system pledges to reduce their impact on the environment.[47,48] Indeed, recent research has explored the safety of repeated washing and then reuse of tube feeding equipment beyond 24 h (reviewed by Osland et al.[49]); however, it was concluded that insufficient evidence exists to support this practice, and other research/guidance also suggests that this is not appropriate.[50–52] Without the ability to wash and reuse, ways in which feeding equipment can be reduced or reused safely are unclear, even though this appears to be occurring in clinical practice, as described above. Therefore, despite the periodic stopping of feeding regimens, feed containers and delivery sets are being reused in the same patient without washing for up to 24 h, thereby reducing the number of feed containers and

delivery sets needed per day. To determine whether this practice is safe from a microbiological perspective, research is required to understand whether bacterial growth could reach unsafe levels when such feeding practices are used. Therefore, this research aimed to assess whether reuse of enteral tube feeding containers and delivery sets over a 24-h period (without washing) using pump bolus feeding, is safe from a microbiological perspective. This laboratory research focused on two objectives, specifically: (1) to practically assess the microbiological safety of the reuse of delivery sets when used with a sterile, ready-to-hang feed, over a 24-h period at room temperature and; (2) to practically assess the microbiological safety of reuse of feed containers and delivery sets used for powdered feeds reconstituted with water, over a 24-h period at room temperature or refrigeration, under several different scenarios.

METHODS

Feed and feed delivery systems

Four different enteral feeding systems were assembled and run either under hygienic controlled conditions or under repeated bacterial challenge conditions, each in triplicate. All four feeding systems used a Flocare Delivery Set (Nutricia Ltd), with feed delivery controlled using a Flocare Infinity Pump (Nutricia Ltd). The enteral feed systems were not washed during the testing and the components remained attached to each other and sealed between feeding sessions. A summary of the four enteral feeding systems are provided in Table 1 and below.

Enteral feeding system 1 (FS1) was a "ready-to-hang," closed system, with a 1-L sterile, multinutrient, fiber-containing commercially available liquid tube feed in a ready-to-hang plastic "Optri-bottle" container (Nutrison Multifibre; Nutricia Ltd). The simulated bolus feeding regimen was 200 ml delivered at 400 ml/h over 30 min, after which the pump was stopped, the delivery set end sealed using the cap provided and the complete sealed enteral feeding system (the container and delivery set) were stored in an incubator at simulated elevated room temperature (24.0 ± 1.0°C) for 5.5 h. The 200-ml feed delivery in 30 min was repeated a further four times with storage in the incubator (as above) for 5.5 h in-between feed deliveries, with a total feeding time of 24.5 h, with all 1 L of feed delivered.

Enteral feeding system 2 (FS2) was a "prepared, powdered," open 1-L container system, with a commercially available pediatric, multinutrient, powdered feed (Neocate LCP; Nutricia Ltd) reconstituted, as per the manufacturer's instructions (870 ml of boiled tap water

TABLE 1 Details of the four enteral tube feeding systems tested.

Enteral feeding system	System type	Starting feed volume and type	Container type	Bolus feed amount delivered in 30 min	Total feed amount delivered in 24.5 h	Storage temperature between bolus feeds
FS1	Ready-to-hang, closed	1 L sterile, multinutrient, fiber-containing liquid tube feed	1 L sterile, sealed, Optri-bottle	200 ml	1 L	24.0 ± 1.0°C
FS2	Prepared, powdered, open	1 L pediatric, multinutrient, powdered feed	1 L container	200 ml	1 L	24.0 ± 1.0°C
FS3	Prepared, powdered, open	200 ml pediatric, multinutrient, powdered feed, prepared five times over 24.5 h	0.5 L container	200 ml	1 L	24.0 ± 1.0°C
FS4	Prepared, powdered, open	200 ml pediatric, multinutrient, powdered feed prepared five times over 24.5 h	0.5 L container	200 ml	1 L	6.0 ± 2.0°C

Note: A closed system is sterilized and sealed until connected to a giving set. Open systems were powdered feeds reconstituted as per the manufacturer's instructions with boiled tap water cooled to 40°C and then added to a feeding container before connection to a giving set.

Abbreviations: FS1, enteral feeding system 1; FS2, enteral feeding system 2; FS3, enteral feeding system 3; FS4, enteral feeding system 4.

cooled to 40°C, 130 g of Neocate LCP powder: 1 L of total volume), in a Flocare 1-L container (Nutricia Ltd). The simulated bolus feeding regimen and storage of the feeding system was as above for FS1.

Enteral feeding systems 3 (FS3) and 4 (FS4) were "prepared, powdered," open 200-ml bolus container systems, with a commercially available, pediatric, multi-nutrient, powdered feed (Neocate LCP; Nutricia Ltd) reconstituted as per the manufacturer's instructions (180 ml of boiled tap water cooled to 40°C, 26 g of Neocate LCP powder: 200 ml of total volume) in a Flocare 0.5-L container (Nutricia Ltd). The simulated bolus feeding regimen delivered 200 ml at 400 ml/h over 30 min, the enteral feeding system was sealed as above and stored for 5.5 h in one of two ways: in an incubator at simulated elevated room temperature (24.0 ± 1.0°C) for FS3 or in a refrigerator at 6.0 ± 2.0°C for FS4. This was repeated a further four times (ie, 200 ml of the feed prepared and delivered in 30 min) with storage as above for 5.5 h in-between feed deliveries, with a total feeding time of 24.5 h and a total of 1 L of feed delivered. For each enteral feeding system set up during feed delivery, the distal end of the delivery set was positioned within a sterile bag to collect the feed, from which samples were collected for microbial growth testing immediately after the 200-ml feed dose had all delivered into the bag.

Bacterial strain preparation

To simulate worst-case bacterial contamination of the enteral feeding systems in the repeated inoculation challenge conditions, seven genera of bacteria originating from food, water, and environmental sources, identified from the literature as major foodborne pathogens with the potential to grow within enteral feeds in a 24-h timeframe when stored at ambient temperatures, were selected.[8,14–16,22,26,34,41,53–58] The strains were sourced either from Leatherhead's own culture collection or National Culture Collections (see Table 2). Three strains of each bacterium were grown, enumerated, and combined into four mixed inocula (see Table 2). The combinations were carefully considered and trialled to minimize the possibility of any antagonistic or synergistic effects, as well as the potential for growth on agar media which could potentially complicate plate reading.

Contamination of the enteral feeding systems

For the repeated inoculation challenge conditions, inoculation of each enteral feeding system was carried out at multiple locations to simulate where contamination was most likely to occur in a real-life scenario due to contamination in the feed, water, or by poor handling technique—that is, at the common "touch points." Inoculation was conducted at a low (but repeatable) level, to simulate contamination at the enteral feeding system contact points by the user (patient/carer/healthcare professional). Inoculation levels were targeted to achieve approximately the following: 100 CFU/swab per organism to inoculate surfaces; 100 CFU/ml per organism introduced via the drug port, and 100 CFU/ml per organism added directly into the reconstituted powdered feed. Three replicate tests were conducted on each of the four enteral feeding systems, per microorganism. For FS1, inoculation occurred at the following points, before the first feeding session had begun: (a) the foil seal of the feed container; (b) the proximal cross-spike connector of the delivery set, and; (c) the outer screw thread of the distal delivery set connector, by dipping a sterile swab into an aliquot of microbial suspension, squeezing the excess from the swab on the side of the aliquot and lightly rubbing the swab over the surface to be inoculated. The drug port was then inoculated during the first feeding session after 100 ml of feed had been delivered, to simulate contamination during administration of a medication very early in the use of the feed system. The feeding pump was stopped, the drug port opened, 100 µl of inoculum pipetted into the port and then the port was flushed with 20 ml of sterile water using an enteral syringe. The port was then capped, and the feeding pump restarted. The distal end of the delivery set was inoculated before the beginning of each feeding session (a total of five times in 24.5 h), to simulate repeat contamination over time, due to the user contaminating the connector when reattaching to the feeding tube before each feeding session. The feed itself was not inoculated in this feeding system, as it is provided sterile and it was not a realistic scenario that the feed would be a source of contamination[20,21,29,33,59] (see Figure 1A). The inoculation of FS2 replicated that of FS1, except that the prepared powdered feed was inoculated directly with 1 ml of bacterial suspension and inverted to distribute the inoculum before the feeding session began, instead of inoculation of the foil seal of the feed container (see Figure 1B). The inoculation of FS3 and FS4 replicated that of FS2, except that each 200-ml dose of prepared powdered feed was inoculated directly with 500 µl of bacterial suspension and inverted to distribute the inoculum before each feeding session began. Direct inoculation of the feed in FS2, FS3, and FS4 simulated bacterial contamination due to poor hygiene during each

TABLE 2 Details of challenge microorganisms and isolation methods used.

Organism combinations	Strain	Original isolation	Strain preparation methods	Enumeration agar and methods
Salmonella species and *Bacillus cereus*	*Salmonella typhimurium*	Dairy ingredient	Strains grown on TSA at 37°C for 24 h. Subbed into TSB for 24 h at 37°C. Samples centrifuged at 3000 *g* for 15 min and washed with SDW, process was repeated three times. Samples stored in SDW at 4°C until day of inoculation.	XLD, 37°C for 24 h
	Salmonella enteritidis NCTC 4444	Human gastroenteritis		
	Salmonella oranienburg	Environmental isolate		
	B cereus	Milk	Grown on lawn plates of TSA at 30°C for 5 days. Spores harvested into SDW and centrifuged as above. Strains heat treated at 80°C for 15 min. Suspensions stored at 4°C until day of inoculation.	MYP. 30°C for 48 h
	B cereus	Milkshake		
	B cereus NCTC 13637	Pasteurized milk		
Escherichia coli and *Staphylococcus aureus*	*E coli*	Milk	Strains grown on TSA at 37°C for 24 h. Subbed into TSB for 24 h at 37°C. Centrifugation and storage as above.	TBX. 44°C for 24 h
	E coli NCTC 10418	Fox feces		
	E coli NCTC 12923	Human feces		
	S aureus subsp. *aureus* ATCC 6538	Lesion		BPA. 37°C for 48 h
	S aureus NCTC 4136	Food poisoning		
	S aureus ATCC 13150	Feces		
Cronobacter sakazakii and *Listeria monocytogenes*	*C sakazakii* NCTC 11467	Human throat	Strains grown on TSA at 37°C for 24 h. Subbed into TSB for 24 h at 37°C. Centrifugation and storage as above.	CCI. 37°C for 24 h
	C sakazakii NCTC 8155	Dried milk powder		
	C sakazakii NCTC 9238	Abdominal pus		
	L monocytogenes	Milk outbreak		OCLA. 37°C for 24–48 h
	L monocytogenes NCTC 11994	Soft cheese		
	L monocytogenes NCTC 7974	Human		
Pseudomonas aeruginosa	*P aeruginosa* NCTC 12924	Outer ear infection	Grown on TSA at 30°C for 24 h. Subbed into TSB for 24 h at 30°C. Centrifugation and storage as above.	Pseudomonas Selective Cetrimide Agar. 30°C for 24 h
	P aeruginosa	Environmental swab		
	P aeruginosa NCTC 7244	Freshwater, well water		

Note: All media sourced from Oxoid Ltd, UK.

Abbreviations: ATCC, American type culture collection; BPA, Baird Parker Agar; CCI, Chromogenic Cronobacter Isolation Agar; MYP, Mannitol Egg Yolk Polymyxin Agar; NCTC, national collection of type cultures; OCLA, Oxoid Chromogenic Listeria Agar; SDW, sterile distilled water; TBX, Tryptone Bile X-Glucuronide Agar; TSA, Tryptone Soya Agar; TSB, Tryptone Soya Broth; XLD, Xylose-Lysine-Desoxycholate Agar.

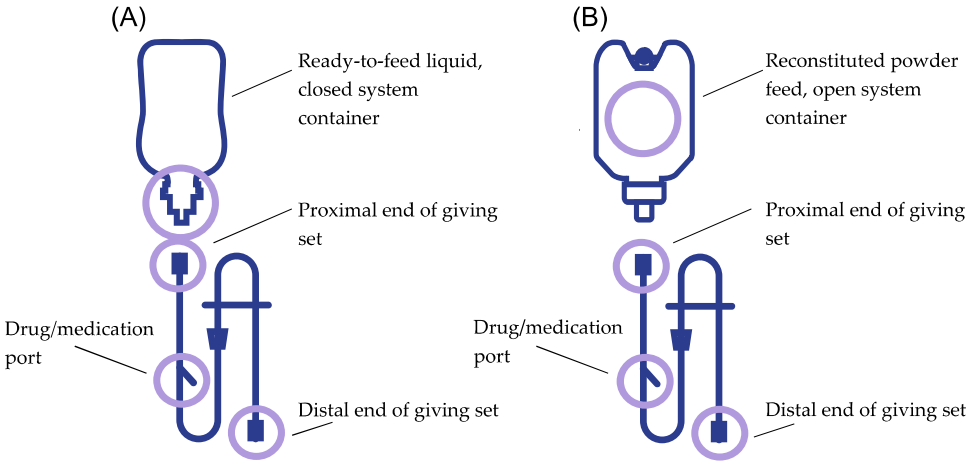

FIGURE 1 Diagram of enteral feeding systems tested representing the ready-to-feed bottle or container and delivery set, with contamination points (circles) shown. Enteral feeding system 1 (FS1) (A) was a "ready-to-feed," closed 1-L system, with a standard, multinutrient, fiber-containing commercially available liquid tube feed in a ready-to-feed plastic container attached to a delivery set. Enteral FS2, FS3, and FS4 (B) were open container systems, with a commercially available pediatric, multinutrient, powdered feed reconstituted as per the manufacturer's instructions, in a container, attached to a delivery set.

preparation of the powdered reconstituted feed (once in FS2, and a total of five times in FS3 and FS4) (see Figure 1B). Otherwise, during preparation and running, all the enteral feeding system equipment and feeds were handled using standard hygienic technique: hands were washed, gloves were worn, and touching of the various contact points (foil seal, cross-spike, delivery set distal end, drug port) was prevented.[24–28] For each of the four enteral feeding systems, hygienic controls were also undertaken where there was no inoculation with challenge bacteria, and standard hygienic technique was used (as above) to simulate how the systems should be prepared and run, according to recommended guidance.

Sampling procedure

For each of the enteral feeding systems, samples for bacterial culture were taken from the 200-ml feed delivered into the sterile stomacher bag at the end of each feeding session, providing five time points (0.5, 6.5, 12.5, 18.5, and 24.5 h). When required, samples were diluted in 9-ml Maximum Recovery Diluent (Oxoid Ltd) prior to plating on appropriate microbiological media. A minimum of two dilutions were plated per sample, with 0.2-ml inoculated volumes on each spread plate. Inoculated samples were tested for the specific challenge bacteria in the inoculum, and hygienic control samples were analyzed for all the challenge bacteria, on each occasion. Individual methods for microbiological enumeration are detailed in Table 2.

Analysis

A lower limit of bacterial count enumeration (CFU/g) was set at 5. As the enteral feeding systems were purposefully inoculated with challenge bacteria, bacterial count enumeration is only reported when appropriate as mean (SD) of the triplicates for all bacterial strains combined; however, bacterial growth over time is reported as the mean change in (Δ) log bacterial growth over time per bacteria compared with baseline (the 0.5-h time point), with significant bacterial growth considered to be >2 Δlog.[25,41,60]

RESULTS

Hygienic control feeding systems

Results from the hygienic control samples of FS1, FS3, and FS4 were below the level of enumeration (<5 CFU/g) for all bacteria, at all time points (see Table 3 and 4). In FS2 most bacteria were consistently below the limit of enumeration throughout the 24.5-h period, except *Bacillus cereus* and *Staphylococcus aureus* levels, which increased from 18.5 h, reaching Δlog of 4.15 and 2.04, respectively, at 24.5 h.

Inoculated challenge feeding systems

FS1

There was no significant growth (>2 Δlog) observed for any of the seven challenge bacteria over time. Overall bacterial levels were on average 100 (SD, 96) CFU/g at the 0.5-h time

TABLE 3 Results of hygienic control enumeration (CFU/g) for the challenge bacteria in FS1 and FS2, over time.

Challenge bacteria	FS1					FS2				
Time, h	0.5	6.5	12.5	18.5	24.5	0.5	6.5	12.5	18.5	24.5
Salmonella	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Bacillus cereus	<5	<5	<5	<5	<5	<5	<5	<5	340	14,000[a]
Escherichia coli	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Staphylococcus aureus	<5	<5	<5	<5	<5	<5	<5	<5	14	110[b]
Cronobacter sakazakii	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Listeria monocytogenes	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Pseudomonas aeruginosa	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

Abbreviations: FS1, enteral feeding system 1; FS2, enteral feeding system 2.

[a]ΔLog 2.53 at 18.5 h, log 4.15 at 24.5 h.

[b]ΔLog 1.15 at 18.5 h, log 2.04 at 24.5 h.

TABLE 4 Results of hygienic control enumeration (CFU/g) for the challenge bacteria in FS3 and FS4, over time.

Challenge bacteria	FS3					FS4				
Time (h)	0.5	6.5	12.5	18.5	24.5	0.5	6.5	12.5	18.5	24.5
Salmonella	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Bacillus cereus	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Escherichia coli	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Staphylococcus aureus	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Cronobacter sakazakii	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Listeria monocytogenes	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Pseudomonas aeruginosa	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

Abbreviations: FS3, enteral feeding system 3; FS4, enteral feeding system 4.

point and 1557 (SD, 4861) CFU/g at the 24.5-h time point, representing a mean Δlog of 0.5 (range, 0–0.69) over the 24.5-h time period.

FS2

No significant growth was observed within the first 6.5 h; however, significant growth of six of the seven bacteria was seen after this time period, with different growth rates depending on the bacteria (see Figure 2). *Salmonella* and *Escherichia coli* had the fastest growth rates, reaching >2 Δlog between the 6.5- and 12.5-h time points, with four of the five remaining bacteria having growth >2 Δlog after the 12.5-h time point. *S aureus* increased by only 1.1 log after 24.5 h.

FS3

No significant growth was observed within the first 10–12 h; however, significant growth (>2 Δlog) of five of the seven bacteria was seen after this time period, with growth occurring at different rates depending on the bacteria (see Figure 3). Significant growth was observed at 12.5 h for *B cereus*, at 18.5 h for *Salmonella*, and at 24.5 h for *C*ronobacter *sakazakii*, *Pseudomonas aeruginosa*, and *E coli*. Growth of *Listeria monocytogenes* and *S aureus* was minimal and not significant (< 2 Δlog), over the 24.5-h period.

FS4

No significant growth was observed for any of the seven challenge bacteria over 24.5 h. Overall bacteria levels were on average 228 (SD 223) CFU/g at the 0.5-h time point and 77 (SD 85) CFU/g at the 24.5-h time point, representing a mean Δlog of −0.08 over the 24.5-h time period.

DISCUSSION

Background bacteria levels in uninoculated hygienic control samples for both ready-to-hang and reconstituted powdered enteral feed systems, were very low. All results

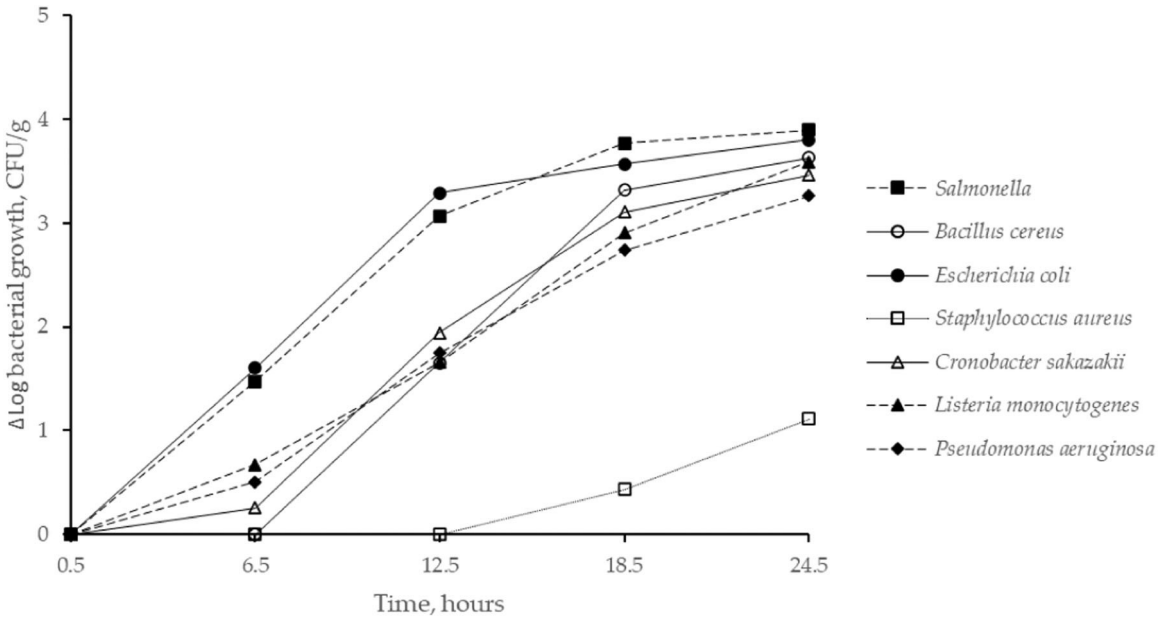

FIGURE 2 Growth of inoculated challenge bacteria over time in enteral feeding system 2 (FS2). 1 L of reconstituted powdered feed, delivered in 200-ml bolus feeds, five times over 24.5 h with feed in the container and delivery set stored at elevated room temperature. Significant Δlog bacterial growth (in CFU/g) of >2 reached after 6.5 h for six of the seven challenge bacteria.

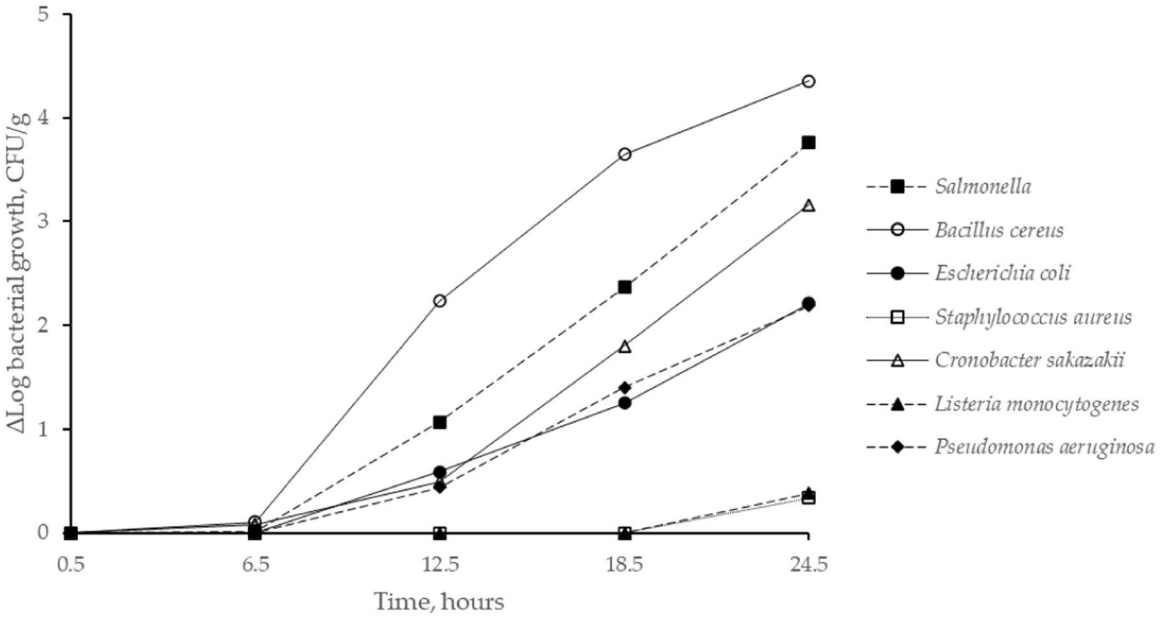

FIGURE 3 Growth of inoculated challenge bacteria over time in enteral feeding system 3 (FS3). 200-ml reconstituted powdered feeds delivered five times over 24.5 h with empty container and delivery set stored at elevated room temperature. Significant Δlog bacterial growth (in CFU/g) of >2 reached after 12.5 h for five of the seven challenge bacteria.

were below the limit of enumeration in three of the four feeding system scenarios for all bacteria tested over 24.5 h. This shows that appropriate hygienic handling technique can prevent bacterial growth in these three enteral feeding system scenarios (FS1, FS3, and FS4) and supports data reported in the literature for use over 24 h.[18,25,33,37] However, the exception was growth of *B cereus* and *S aureus* after 18.5 h in FS2, where the 1-L of reconstituted feed was stored between 200-ml feeding sessions at elevated ambient temperature for 24.5 h. This feeding system design is not in line with guidance on the maximum hanging time of 4 h for reconstituted

feeds,[32,38,39] so could be considered an unrealistic scenario. However, these results show that with a hygienic handling technique, bacterial growth is limited until 18.5 h, although the 4-h hanging time guidance is appropriate and should be followed.

Of the bacterial inoculation challenges conducted with storage at elevated ambient temperature in FS1, FS2, and FS3, the sterile, 1-L, ready-to-hang system FS1 showed the smallest increase in microbial levels, as has been shown previously.[25,33,37] As the feed itself was sterile and sealed and it was not directly inoculated in this feeding system (only the foil seal and the proximal cross-spike connector, which would allow introduction of bacteria into the feed), these results were not unexpected; however, results across the triplicate tests were more variable because of the inoculation method on to the foil seal surface, producing larger SD than those seen in the other feeding systems. This feeding system closely mirrored a real-life enteral feeding scenario, with contamination at key touch points, even though the bacterial contamination level was considerably higher than would be likely in an acute or home care setting. This feeding regimen with boluses of 200 ml delivered at 400 ml/h over 30 min, once every 5 h over a 24-h period, also mirrored bolus feeding regimens[42–46] or the repeated disruption of continuous feeding due to clinical care and interventions, or activities of daily life which occur in all healthcare settings. Although overall these results are very encouraging, bacterial growth may still be possible if a particularly serious contamination event were to occur. However, with appropriate precautions in place to reduce contamination by the user to an acceptable level, it should be possible to reuse a delivery set that is capped, attached to a ready-to-hang bottle, and stored at ambient temperature over a 24-h period. These results highlight the importance of reusable caps on delivery sets to ensure they can be safely sealed while stored. Storing the ready-to-hang bottle and delivery set under refrigeration conditions would give more confidence, as results from FS4 demonstrate (see below). If this evidence is used to change clinical practice it has considerable potential to reduce delivery set usage and therefore reduce costs to healthcare systems and plastic wastage.

Results from the bacterial inoculation challenges in FS2 produced the greatest increases in bacterial growth, with significant growth occurring after 6.5 h. The initial inoculation into the feed itself and larger feed volume (1 L) incubated at elevated ambient temperature between feeding sessions, encouraged greater bacterial growth levels overall. As both the hygienic controls and the inoculated challenge for this feeding system showed significant bacterial growth, it is clear that use of this enteral feeding system over 24 h is most likely to encourage bacterial growth, especially if there is poor hygienic handling technique. Although this feeding system is equivalent to the feeding regimen of FS1, the key difference is the use of a powdered feed that needed to be prepared with water by the user instead of a sterile, sealed ready-to-hang liquid feed. This reconstituted powdered feed was directly contaminated with bacteria to replicate poor preparation technique, with considerable "no flow" time in-between feeding sessions[56] likely accounting for the significant bacterial growth rates. However, this scenario could be considered unrealistic in comparison with current clinical practice and guidelines, that state a maximum hanging time for reconstituted powdered feeds of 4 h.[32,38,39] Importantly, no significant bacterial growth was observed within 4 h in this feeding system. Therefore, if the maximum hanging time guidance of 4 h is followed and effective hygienic handling technique is used, bacterial growth should be minimal in this feeding system; however, reuse of containers or delivery sets beyond 6.5 h in this feeding scenario would not be recommended.

Results from the bacterial inoculation challenge in FS3 showed significant bacterial growth occurring at 12.5 h. In this feeding system, 200-ml boluses of powdered feed were prepared fresh for each feeding session and run at 400 ml/h over 30 min, with the container and delivery set containing residual feed and stored at elevated ambient temperature between feeding sessions. In this scenario the feed container was not emptied of residual feed, cleaned, or rinsed, and the delivery set remained attached to the container during storage, detached only to allow addition of the next feed. The feed was inoculated with bacteria at the point of each feed preparation. Hanging time guidance of maximum 4 h for reconstituted feeds was adhered to in this feeding system. As there was no significant bacterial growth in hygienic controls for this feeding system and significant bacterial growth in inoculation challenges did not occur until 12.5 h, it should be possible to reuse this system with a delivery set that is capped and attached to a sealed container, stored at ambient temperature over a 10-h period, with appropriate techniques in place to reduce contamination by the user to an acceptable level. Therefore, if this evidence is used to change clinical practice, it has considerable potential to reduce delivery set and container usage and therefore reduce costs to healthcare systems and plastic wastage.

The bacterial inoculation challenge in FS4 was a repeat of FS3, but with the container and delivery set containing residual feed stored at refrigeration temperature between feeding sessions, and there was no significant bacterial growth in this feeding system

throughout the 24.5-h time period. When compared with FS3, in which only the storage temperature differed, these results demonstrate that refrigerated storage at 4–8°C is an effective control to prevent growth of the challenge bacteria over a 24-h period, with a reused feed container and delivery set. Therefore, with appropriate techniques in place to reduce contamination by the user to an acceptable level, in this enteral feeding scenario it should be possible to reuse this system with a delivery set that is capped and attached to a sealed container stored at refrigeration temperature over a 24-h period. However, appropriate refrigerated storage needs to be considered, especially in clinical ward settings, to prevent cross-contamination between systems. If this evidence is used to change clinical practice, it has considerable potential to reduce delivery set and container usage and therefore reduce costs to healthcare systems and plastic wastage.

There are a few limitations of this research design. This research was undertaken in vitro, under laboratory conditions, to mirror real-life clinical practice scenarios; however, bacterial contamination of enteral feeding systems being used by patients is not possible or ethical because of the potential harm that could be caused to patients. The methodology employed is based on many studies published in the literature,[10,20,21,56] which have shaped the design and safe use of enteral feeding administration equipment over the last 40 years, and so the data produced can be considered to be robust and transferable to real-life clinical practice. However, other methods used previously[21,23,24,37,56,59] to swab a patient's enteral feeding equipment or take samples of feed from systems being used in clinical practice have been shown to produce data for similar evaluations of bacterial growth, although these data do not appear to differ significantly from that produced in laboratory tests.[20,23,59] The hygienic control testing provided evidence for the inherent presence and growth of the seven bacteria under investigation only, and the inoculated challenges provided data on the growth of the seven challenge bacteria only. Future similar work with patients to assess and identify the bacterial presence and growth risk when reusing delivery sets and containers over 24 h should be undertaken to further explore these findings. In these feeding systems, considerable repeated bacterial contamination was introduced to the enteral feeding systems from seven common bacterial pathogens at 50 CFU/ml per organism, similar to that used previously.[20,21,56] This could be considered an unrealistically high level of bacterial contamination that would be highly unlikely to occur in clinical practice from feed preparation or contaminated hands/surfaces.[20] However, contamination levels needed to be at high enough levels to imitate a worst-case scenario of bacterial contamination and ensure a comprehensive and robust design to assess bacterial growth. Lower levels of contamination could be challenged as insufficient and would have affected repeatability. Similarly, four combinations of bacterial strains were used, and the mix of strains could have resulted in impedance of bacterial growth, yet the strain combinations were carefully considered and trialled to minimize the possibility of any antagonistic or synergistic effects. The seven bacteria were chosen following an extensive review of the literature to establish the common bacterial pathogens known to cause symptoms or diseases in humans and that have been studied in the associated literature.[22,23,26,34,40,56–58] Other commonly found strains of bacteria have also been studied in enteral feeding systems previously (ie, *Klebsiella* spp[20,23] or *Streptococcus* spp).[24,59] The seven bacteria chosen in this research were considered representative and appropriate, with evidence of growth rates in these types of media. Further bacteria may have different growth rates in enteral feeding systems, and further research could explore the bacterial growth rates of other bacteria and/or other strains of the bacteria utilized here. Similarly, this study did not assess the potential for bacterial growth to cause gastrointestinal or other symptoms in patients. Another potential limitation is the two main types of enteral feed that were investigated in this study, namely a sterile, standard, multinutrient, fiber-containing liquid tube feed and a pediatric, amino acid-based, multinutrient, powdered feed. In the literature relatively minor variations in energy and nutrient density appear to have little effect on bacterial growth rates[21,22,33]; therefore, these results should be reproducible in other commonly used commercially available liquid or powdered enteral tube feeds. Blended (homemade) feeds have not been tested in this study[32,40,53,61–63] and therefore, these results could not be extrapolated for blended diet feeds, with further research needed before reuse of containers and delivery sets can be considered with this feed type. FS2, FS3, and FS4 used a powdered feed prepared with boiled tap water cooled to 40°C as per the manufacturer's instructions; however, the quality and availability of boiled water can vary between and within countries, as can guidance on what types of water can be used in enteral tube feeding. Different types of water were not tested in this research, and as water can be a source of bacterial contamination, this needs to be a consideration in the implementation of any changes to clinical practice.[64] Similarly, not all variations of enteral feeding regimens, that is, volume of bolus provided, rate provided and time between feeding sessions, have been explored here; however, from the

literature and anecdotal feedback from UK healthcare professionals, FS1, FS3, and FS4 are representative of 24 h feeding regimens typically used in clinical practice. Furthermore, the controlled temperature scenarios of simulated elevated room temperature (24.0 ± 1.0°C) and refrigeration at 6.0 ± 2.0°C, may not be representative of storage temperatures in varying geographical locations, climates and seasons, which should be taken into consideration regarding the generalizability of these findings. The use or reuse of delivery sets and containers in a single patient in 24 h may not be appropriate for all patient groups, especially those who are immunocompromised, have a sensitive gut or are complex, and in certain acute hospital settings (that is, intensive care), and any changes to clinical practice should always be thoroughly risk assessed, ensuring compliance with infection control guidance and procedures. Furthermore, surveys of healthcare professionals to determine the incidence of suspected microbial infection from enteral feeding systems, the understanding of guidance and manufacturers information, and current practice with regard to interpretating single-patient-use and if delivery sets are used over 24 h, may provide further insights into how plastic equipment use can be reduced.

Overall, these results show that with hygienic handling technique there is limited bacterial growth within the modelled scenarios presented here with reuse of delivery sets and containers, that refrigeration of the delivery set and container between feeding sessions reduces bacterial growth risk, and that using small bolus volumes of reconstituted powder feed reduces bacterial growth risk. These results indicate that reuse of delivery sets and feed containers in a single patient over a 24-h period is possible from a microbiological perspective, if they are refrigerated at <8°C between uses, even when contamination of the feed and delivery set occur. Research to further explore the safe reuse of enteral feeding administration equipment is needed to confirm these results with the aim to deliver savings for healthcare systems and environmental benefits from reduced plastic waste.

AUTHOR CONTRIBUTIONS

Conceptualization and methodology: Gary P. Hubbard, Johanna Van Wyk, Louise Grinyer, Richard Onley, and Rebecca J. Stratton. Investigation and acquisition of data: Gary P. Hubbard, Johanna Van Wyk, Louise Grinyer, and Richard Onley. Formal analysis: Gary P. Hubbard and Richard Onley. Writing, specifically original draft preparation: Gary P. Hubbard. Writing, specifically reviewing, and editing: Gary P. Hubbard, Johanna Van Wyk, Laura Forwood, Richard Onley, Louise Grinyer, Sean White, Carole-Anne Fleming, Janet Baxter, and Rebecca J. Stratton. Visualization: Gary P. Hubbard and Johanna Van Wyk. Supervision: Gary P. Hubbard, Johanna Van Wyk, and Rebecca J. Stratton. Project administration: Gary P. Hubbard, Johanna Van Wyk, Louise Grinyer, and Richard Onley. Funding acquisition: Gary P. Hubbard, Johanna Van Wyk, and Rebecca J. Stratton.

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CONFLICT OF INTEREST STATEMENT

Gary P. Hubbard, Johanna Van Wyk, Louise Forwood, and Rebecca J. Stratton are employees of Nutricia Ltd. Sean White is a member of Nutricia tube feeding Advisory Board. No other conflicts of interest are declared.

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