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Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii*, *Salmonella* serovars and other *Enterobacteriaceae*

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ABSTRACT

WHO (2007) recommended that to reduce microbial risks, powdered infant formula should be reconstituted with water at temperatures >70 °C, and that such feeds should be used within 2 h of preparation. However, this recommendation does not consider the use of enteral feeding tubes which can be in place for more than 48 h and can be loci for bacterial attachment. This study determined the extent to which 29 strains of *Cronobacter sakazakii*, *Salmonella* serovars, other *Enterobacteriaceae* and *Acinetobacter* spp. can adhere and grow on enteral feeding tubes composed of polyvinyl chloride and polyurethane. The study also included silver-impregnated tubing which was expected to have antibacterial activity.

Bacterial biofilm formation by members of the *Enterobacteriaceae* was *ca.* 10^5–10^6 cfu/cm after 24 h. Negligible biofilm was detected for *Acinetobacter* gensp. 13; *ca.* 10 cfu/cm, whereas *Cr. sakazakii* strain ATCC 12868 had the highest biofilm cell density of 10^7 cfu/cm. Biofilm formation did not correlate with capsule production, and was not inhibited on silver-impregnated tubing. Bacteria grew in the tube lumen to cell densities of 10^7 cfu/ml within 8 h, and 10^9 cfu/ml within 24 h. It is plausible that *in vivo* the biofilm will both inoculate subsequent routine feeds and as the biofilm ages, clumps of cells will be shed which may survive passage through the neonate's stomach. Therefore biofilm formation on enteral feeding tubes constitutes a risk factor for susceptible neonates.

1. Introduction

In 2004, following international concern over the microbiological safety of powdered infant formula, the FAO/WHO (2004) undertook a risk assessment of the organisms associated with neonatal infections and powdered infant formula. They categorised *Cronobacter* spp. (*Enterobacter sakazakii*) and *Salmonella* serovars as 'clear evidence of causality' (Category 1), and other *Enterobacteriaceae* were deemed 'causality plausible, but not yet demonstrated' (Category 2). *Acinetobacter* spp. was added to the latter group by FAO/WHO in 2006. Members of the *Cronobacter* genus are associated with infections of immunocompromised individuals especially neonates (Forsythe, 2005; Iversen et al., 2008). Bowen and Braden (2006) considered 46 cases of *Cronobacter* infections in neonates. They reported that the symptoms of very low birth weight neonates (age of onset *ca.* 1 month) tend to be bacteraemia, whereas those of birth weight *ca.* 2000 g suffered from meningitis and an onset age of a few days. Due to the association of some *Cronobacter* cases with contaminated powdered infant formula, this product has come under considerable attention with regard to its microbial safety and various detection methods have been developed (Fanning and Forsythe, 2008). It is expected that intrinsic contamination of powdered infant formula will decrease due to the subsequent improved industrial processes and microbiological criteria (Codex Alimentarius Commission, 2008a,b). However, despite the long established methods for the detection of *Salmonella* serovars, along with monitoring and control measures, between 1985 and 2005 there were at least 6 outbreaks of salmonellosis associated with infant formula (Cahill et al., 2008). These cases involved *ca.* 287 infants in total. More recently, there have been two further outbreaks of *Salmonella* associated with infant formula in France and Spain involving 14 and 23 cases (Jourdan et al., 2008; Soler et al., 2008). Therefore routes of neonatal infection *via* infant formula need to be reconsidered in order to implement any necessary additional control measures.

The FAO/WHO meetings in 2004 and 2006 stressed that multiplication of *Cronobacter* spp., *Salmonella* serovars, and Category 2 organisms following reconstitution equated to increased risk of infection. Subsequently the WHO (2007) recommended that to reduce microbial risks, powdered infant formula should be reconstituted with water at temperatures >70 °C, and that such feeds should be used within 2 h of preparation. Three reported outbreaks of *Cronobacter* spp. have involved possible temperature abuse of reconstituted feed, allowing bacterial overgrowth to occur (Himelright et al., 2002; Van Acker et al., 2001; Caubilla-Barron et al., 2007). Nevertheless, the WHO recommendation does not consider the use of enteral feeding tubes which can be in place for more than 48 h and can be loci for bacterial attachment (Mehall et al., 2002). Since the tubes

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are at body temperature and contain nutrients (infant formula), it is reasonable to anticipate that bacteria will multiply in the tube and contaminate subsequent feeds. It is already known that *Cronobacter* spp. can form biofilms on plastic surfaces (Iversen et al. 2004; Kim et al. 2006).

Kim et al. (2006) reported that *Cronobacter* spp. attached to enteral feeding tubes at ambient temperatures, and as the biofilm aged the cells became detached. The detached cells will be in clumps, and may be protected from the stomach acidity due to capsule formation. Consequently they may pass into the infant intestines and constitute a risk to neonatal health. The attachment of *Acinetobacter* spp., and *Enterobacteriaceae*, including *Cronobacter* spp. and *Salmonella* serovars, to enteral feeding tubes at body temperature (as occurs with enteral feeding of neonates on an intensive care unit) has not been considered in detail previously. This study has determined the attachment of *Enterobacteriaceae* and *Acinetobacter*, organisms associated with neonatal infections, to enteral feeding tubes. The study included silver-impregnated tubing which is expected to have antibacterial activity.

2. Materials and methods

2.1. Bacterial strains

Bacterial species included 29 strains of *Cr. sakazakii*, *Salmonella* serovars, *Enterobacter cloacae, Citrobacter freundii, Cit. koseri, Pantoea* spp., *Escherichia coli, Esch. hermanii, Esch. vulneris, Klebsiella oxytoca, K. pneumoniae, Hafnia alvei, Serratia marcescens,* and *Acinetobacter* species. The bacterial strains and their sources are summarised in Table 1. Where possible strains were chosen on the basis of isolation source (powdered infant formula, dairy products, or enteral feeding tubes). Additionally, in order to investigate any correlation between capsule production and biofilm formation, *Cr. sakazakii* strains were chosen according to the amount of capsule production on milk agar; Table 2.

Table 1
Description of strains used in this study.

Organism	Source
Cr. sakazakii NCTC 11467 ^{T}	Infant clinical
Cr. sakazakii ATCC 12868	Unknown
Cr. sakazakii 1	Powdered infant formula
Cr. sakazakii 2	Powdered infant formula
Cr. sakazakii 3	Powdered infant formula
Cr. sakazakii 4	Enteral feeding tube
Cr. sakazakii 5	Powdered infant formula
Cr. sakazakii 6	Powdered infant formula
Cr. sakazakii 7	Powdered infant formula
Cr. sakazakii 8	Raw material
Cr. sakazakii 9	Unknown
Cr. sakazakii 10	Environmental
Sal. enterica serovar Anatum	Dairy product
Sal. Give	Dairy product
Sal. Kedougou	Clinical isolate
Esch. coli K12	Unknown
Esch. hermanii	Milk powder
Esch. vulneris	Powdered infant formula
Cit. freundii	Powdered infant formula
Cit. koseri	Powdered infant formula
Ent. cloacae	Powdered infant formula
Ent. hormaechei	Enteral feeding tube
Hafnia alvei	Dairy unit
Klebsiella oxytoca	ATCC 41365
K. pneumoniae	Milk powder
Pantoea spp.	Powdered infant formula
Ser. marcescens	Enteral feeding tube
Acinetobacter gensp. 13	Cheese
A. calcoaceticus	Cheese

2.2. Tubing

Three types of tubing were evaluated for supporting bacterial biofilm formation; polyvinylchloride (PVC, gauge 3.5), polyurethane (PU, gauge 5), and silver-impregnated flexilene plastic. The first 2 types of tubing are used as enteral feeding tubes. The silver-impregnated tubing is not used as enteral feeding tube, but was evaluated for its potential antibacterial activity.

2.3. Bacterial capsule formation determination

Bacterial capsule production on milk agar was determined as previously described (Caubilla-Barron and Forsythe, 2007). Three grams of agar (LP0011, Oxoid Ltd., Basingstoke, UK) and 0.4 g of ammonium sulphate were dissolved in 40 ml of distilled water. After autoclaving (121 °C for 15 min) the mixture was combined with 200 ml of warm (55 °C) sterile whey-based ready to feed infant formula milk and dispensed into Petri dishes. Plates were inoculated and incubated for 24 h, at 37 °C. Colony morphology was compared with *Cr. sakazakii* strains NCTC 11467 ^{T} (non-capsulated) and ATCC 12868 (capsulated) as previously described by Iversen et al. (2004).

2.4. Biofilm formation determination

Biofilm formation was determined using the impedance technique (Silley & Forsythe, 1996) with the Rapid Automated Bacterial Impedance Technique (RABIT™, Don Whitley Scientific Ltd., UK). Bacterial inocula were grown at 37 °C overnight without shaking in sterile infant formula. Ten millilitre volumes of sterile infant formula containing five 1 cm lengths of tubing (sterilised by immersion in 70% ethanol) were inoculated with 0.1 ml of an overnight culture. After incubation for 24 h at 37 °C without shaking, the infant formula was aseptically removed and an aliquot retained for viable count determination on TSA (Merck 1.05458). The five 1 cm pieces of tubing were washed by aseptically transferring to 10 ml of sterile saline followed by shaking for 2 min. This procedure was repeated a further two times. Each piece of tubing with remaining bacterial biofilm was transferred to a sterile impedance tube containing 2 ml of Brain Heart Infusion broth (BHI, Merck 1.10493), and placed in the impedance instrument (RABIT™) within 10 min of preparation. Therefore, there were five replicates for each bacteria-tubing combination. The impedance calibration curves for each bacterial strain were established using plate counts on TSA at 37 °C for 24 h. Bacterial biofilm values were expressed as viable count ($\log_{10}$ cfu) cm^{-1} of tubing.

2.5. Growth rate determination

To determine the growth rate of the organisms, 0.1 ml decimal dilutions of overnight cultures were inoculated into 2 ml infant formula (whey-based) and incubated at 37 °C. The doubling time was calculated from the growth response calibration curve as previously described (Silley & Forsythe 1996; Iversen et al. 2004).

2.6. Bacterial attachment to enteral feeding tube wall and inoculation of lumen contents

To mimic hospital practices with respect to frequency of feeding, enteral feeding tubes were inoculated with *Cr. sakazakii* strains ATCC 12868 and 4 followed by flushing at regular intervals with sterile infant formula. The bacterial strains were grown beforehand at 37 °C in liquid infant formula for 18 h, and then diluted to 10^5 cfu/ml in sterile saline. The culture was syringed through PVC enteral feeding tubes, which were then incubated at 37 °C. At 2 hourly intervals, the residual lumen liquid was displaced into a sterile Eppendorf tube by attaching a sterile syringe to the tube and pushing air through the tubing. Afterwards, 10 ml sterile liquid formula was syringed through

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Table 2
Capsule production and biofilm formation of *Enterobacteriaceae* and *Acinetobacter* spp. on enteral feeding tubes, and silver-impregnated tubing.

Organism	Capsule production	Polyvinylchloride (log_{10} cfu/cm)			Polyurethane (log_{10} cfu/cm)			Silver-impregnated (log_{10} cfu/cm)		
		Av	Max	Min	Av	Max	Min	Av	Max	Min
Cr. sakazakii NCTC 11467 ^{T}	−	5.7	6.3	4.0	6.2	6.6	5.2	6.3	6.8	5.1
Cr. sakazakii ATCC 12868	+++	4.6	4.8	4.3	6.3	6.7	5.6	6.4	6.5	6.1
Cr. sakazakii 1	+++	6.3	6.4	5.8	5.6	5.9	5.3	7.4	7.7	7.0
Cr. sakazakii 2	+++	5.5	5.7	4.9	5.9	6.1	5.5	7.0	7.0	6.5
Cr. sakazakii 3	+++	5.3	5.6	5.0	6.2	6.7	5.8	6.5	6.8	6.1
Cr. sakazakii 4	+++	5.0	5.4	4.5	6.7	6.7	6.7	6.1	6.3	5.1
Cr. sakazakii 5	++	5.6	5.8	5.3	5.5	5.6	5.1	5.3	5.4	5.1
Cr. sakazakii 6	++	4.6	4.9	4.0	5.3	5.5	4.7	6.0	6.4	5.0
Cr. sakazakii 7	+	5.9	6.2	5.2	6.4	6.9	5.7	6.4	6.9	5.7
Cr. sakazakii 8	+	4.6	4.9	4.5	5.3	5.5	5.0	5.4	5.6	5.1
Cr. sakazakii 9	−	5.3	5.5	4.4	5.9	6.1	5.7	6.1	6.5	4.6
Cr. sakazakii 10	−	6.2	6.6	5.5	6.9	7.0	6.6	6.9	7.2	6.5
Sal. Anatum	+	6.6	6.9	5.3	6.6	6.8	6.3	6.7	7.1	5.9
Sal. Give	+	5.3	5.7	5.0	6.9	7.3	6.3	6.7	7.0	6.3
Sal. Kedougou	++	5.7	5.9	5.2	6.0	6.2	5.5	5.6	5.7	5.2
Esch. coli K12	−	4.4	4.7	3.9	4.6	4.6	4.6	4.4	4.5	4.2
Esch. hermanii	−	6.3	6.6	6.1	6.7	6.8	6.6	6.7	6.9	6.1
Esch. vulneris	−	6.4	6.6	5.9	6.4	6.8	5.8	6.5	6.9	6.0
Cit. freundii	−	5.1	5.4	4.4	5.9	6.1	5.5	5.9	6.3	5.4
Cit. koseri	−	5.1	5.3	4.8	6.0	6.2	5.8	6.0	6.1	5.7
Ent. cloacae	++	5.1	5.5	3.4	5.9	6.0	5.6	3.7	3.9	3.3
Ent. hormaechei	+	5.7	6.0	4.9	6.4	6.7	5.9	5.6	5.7	5.5
H. alvei	−	4.9	5.0	4.7	5.1	5.2	4.8	5.6	5.8	5.0
K. oxytoca	−	5.2	5.5	4.7	5.4	5.8	4.9	6.0	6.5	5.4
K. pneumoniae	+	5.6	5.7	5.2	5.2	5.4	5.0	5.9	6.2	5.7
Pantoea spp.	++	5.6	5.9	5.2	5.6	5.6	5.4	6.2	6.3	6.2
Ser. marcenscens	+	5.8	4.1	6.2	5.9	4.0	6.4	6.2	6.4	5.4
Acinetobacter gensp.13	−	4.1	4.0	4.1	2.9	3.3	1.6	4.8	5.3	4.1
A. calcoaceticus	+	1.3	1.7	0.7	0.5	1.0	−2.0	0.6	1.0	0.1

the tubing which was then reincubated at 37 °C. The number of bacteria in the lumen liquid, and attached to the tube wall was enumerated as described above.

2.7. Scanning electron microscopy of Cr. sakazakii attachment to enteral feeding tube

PVC tubing which had been incubated with *Cr. sakazakii* strain ATCC 12868 overnight, as described above, was fixed using 3% gluteraldehyde prepared in a 0.1 M phosphate buffer. The tubing was cut into representative 1 cm lengths and dissected longitudinally to expose the inner surface. The pieces were then washed in 0.1 M phosphate buffer, post fixed in 1% (w/v) osmium tetroxide, dehydrated through a gradual series of alcohols up to 100% alcohol and then treated with hexamethyldisilazane (100%) for 5 min. The air-dried pieces were then attached to aluminium stubs, sputter-coated with gold and examined using a Stereoscan S250 Mark III SEM at 10–20 KV.

3. Results

Capsule formation by 29 strains of various *Enterobacteriaceae* genera and *Acinetobacter* spp. was determined by colony appearance on milk agar; Table 2. *Cr. sakazakii* strains ATCC 12868, 1, 2, 3 and 4 had the most mucoid appearance indicating capsular material production. Strains 1–3 had been isolated from powdered infant formula (Table 1), and strain 4 from an enteral feeding tube on a neonatal intensive care unit. Less capsular material was produced by the *Cr. sakazakii* strains 5–8. The dry colony appearance of the *Cr. sakazakii* type strain NCTC 11467 ^{T}, 9 and 10 indicated no capsular material was produced. *Cr. sakazakii* NCTC 11467 ^{T} was a clinical isolate from a child's throat, and the remaining two were from powdered infant formula. *Sal.* Kedougou, *Ent. cloacae* and *Pantoea* spp. produced mucoid colonies on milk agar. Whereas, the *Salmonella* serovars Anatum and Give, *K. pneumoniae* and *A. calcoaceticus* colonies only had a slight mucoid appearance. The cells densities of overnight cultures in infant formula ranged from 10^8 to 10^{10} cfu/ml. *Cr. sakazakii* and *Salmonella* serovars, most other *Enterobacteriaceae* and *Acinetobacter* spp. had doubling times of 22–27 min. The slowest growing organism was *H. alvei* with a doubling time of 38 min.

Each biofilm cell density measurement was obtained using five replicates. As previously reported (Iversen et al. 2004), the biofilm cell density value varied notably between replicates, and hence minimum and maximum values are indicated in Table 2. The 12 strains of *Cr. sakazakii* grew as biofilms on the three types of tubing; PVC, PU, and silver-impregnated flexelene. The highest biofilm level was recorded with *Cr. sakazakii* strain 1 on the flexelene tubing at 7.7 log_{10} cfu/cm. There was no correlation between biofilm cell density, and capsule formation on milk agar; Table 2. Non-mucoidal *Cr. sakazakii* strains 9 and 10 produced biofilms at levels which were indistinguishable for the other *Cr. sakazakii* strains. *Sal.* Anatum, *Sal.* Give and *Sal.* Kedougou produced biofilms on the three types of tubing at levels similar to *Cr. sakazakii*; *ca.*10^5–10^7 cfu/cm.

Of the remaining *Enterobacteriaceae*, *Esch. coli* K12 had the lowest level (10^4 cfu/cm) of biofilm formation on all three types of tubing. *Esch. hermanii* and *Esch. vulneris*, which also showed no capsule production on milk agar plates, had biofilm levels approximately 100-fold higher at 10^6 cfu/cm. In general, across all the bacterial species, the level of biofilm formation on the silver-impregnated tubing was higher than that on the PVC and PU enteral feeding tubes. There was no indication of antibacterial activity by the silver-impregnated flexelene tubing, except against *Ent. cloacae* which showed lower biofilm formation on the silver-impregnated tubing. Biofilm formation by *Acinetobacter* gensp. 13 was low, and was negligible for *A. calcoaceticus*; Table 2.

Two strains of *Cr. sakazakii* (ATCC 12868 and 4) were chosen to study the time course of bacterial attachment to PVC tubing. The former is a well studied capsule-producing strain and the latter was

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originally isolated from an enteral feeding tube on a neonatal intensive care unit. PVC tubing was in use at the neonatal intensive care unit from which *Cr. sakazakii* strain 4 had been isolated. To simulate hospital practices, 37 °C incubated PVC enteral feeding tubes were gently flushed with fresh sterile formula every 2 h, and the lumen contents collected. The number of bacteria on the tube wall and the residual liquid in the tube lumen was determined using the impedance method. Fig. 1 shows bacterial numbers on the tubing wall was 10^4–10^5 cfu/cm by 8 h, and 10^4–10^6 cfu/cm after 24 h. *Cr. sakazakii* strain 4 produced higher biofilm cell density than strain ATCC 12868. The organisms also multiplied in the fresh liquid feed of the tube lumen to 10^7 cfu/ml by 8 h, 10^8–10^9 cfu/ml with 24 h and had a doubling time of 22 min. Fig. 2 is a scanning electron microscope image of *Cr. sakazakii* ATCC 12868 biofilm on PVC neonatal enteral feeding tubing after 24 h incubation. Cells of *Cr. sakazakii* in clusters can be seen within a matrix of liquid feed material.

4. Discussion

Enterobacteriaceae are able to attach to inert surfaces and grow, resulting in biofilm formation (Murga et al. 2001; Thompson et al. 2006; Zogaj et al. 2003). Therefore, it is plausible that bacteria attach *in vivo* to neonatal enteral feeding tubes, and grow to high cell densities. *Cr. sakazakii* strain 4, *Ent. hormaechei* and *Ser. marcescens* used in this study were originally isolated from discarded enteral feeding tubes in a neonatal intensive care unit and therefore emphasize the clinical significance of this work (Table 1). Enteral feeding tubes are used to feed low birth weight infants on neonatal intensive care units, and may be left *in situ* for several days. The tubes will be at 37 °C, and receive fresh nutrients (infant feed) at every 2–3 h according to the neonate's feeding regime (Hurrell et al., in press). As shown in this study *Enterobacteriaceae,* such as *Cr. sakazakii* and *Salmonella* serovars, are able to colonize enteral feeding tubes to high cell densities (ca. 10^5–10^6 cfu/cm) which will lead to contamination of subsequent feeds (Table 2 and Fig. 1).

Previously, Iversen et al. (2004) reported *ca.* 2 log variations in the viable counts between replicates of *Cr. sakazakii* biofilms on polycarbonate, latex and silicon surfaces using the same impedance technique. This large variation was also seen in our study of a wider range of *Enterobacteriaceae* and *Acinetobacter* spp. and therefore the average, minimum and maximum biofilm cell densities per organism are presented in Table 2. This variation is probably due to irregular attachment and growth on the tubing and the fragile nature of the biofilm which can be disrupted on washing and transferring to the impedance tubes. This serves to emphasize that a bacterial biofilm on a neonatal enteral feeding tube is unstable and will release clumps of bacteria.

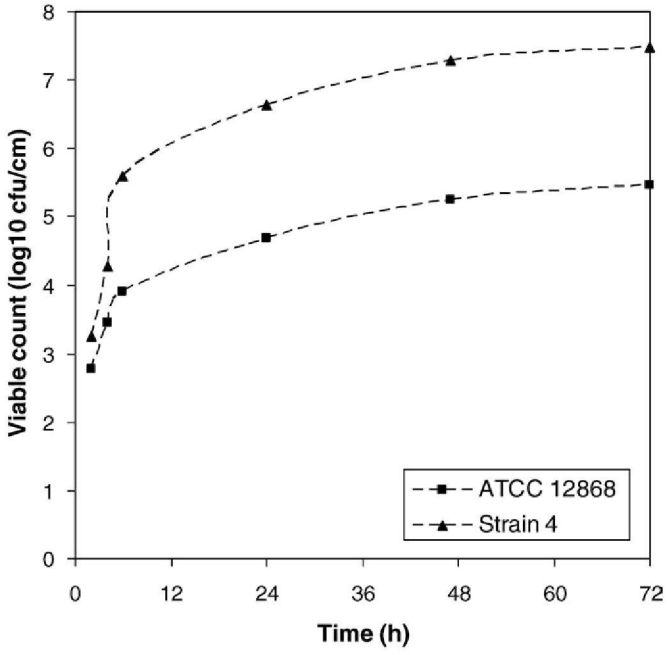

Fig. 1. *Cr. sakazakii* biofilm formation on enteral feeding tubes.

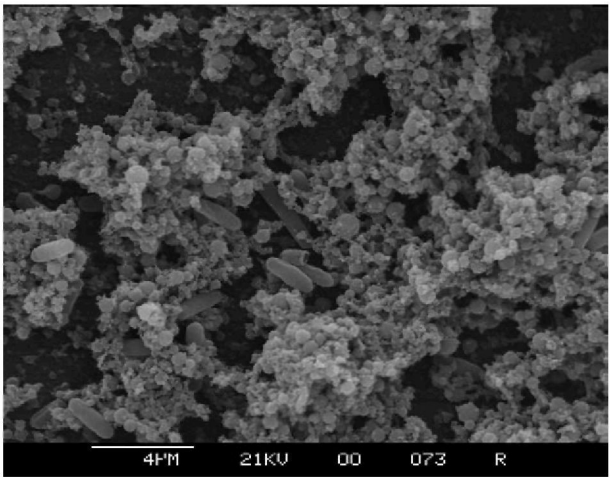

Fig. 2. Electron micrograph of *Cr. sakazakii* ATCC 12868 attached to PVC enteral feeding tube. Tubes had been incubated with inoculated infant formula for 24 h before preparation. *Cr. sakazakii* cells can be seen within the matrix of the infant formula material.

There was no correlation between biofilm cell density, and capsule formation on milk agar for the 29 strains studied; Table 2. For example, *Cr. sakazakii* strains NCTC 11467 ^{T} and 10 did not produce any capsule on milk agar plates, yet produced more biofilm on PVC than *Cr. sakazakii* strains ATCC 12886, 4, and 6. Similarly on PU and silver-impregnated tubing, *Cr. sakazakii* NCTC 11467 ^{T} and strain 10 produced more biofilm than most capsulated *Cr. sakazakii* strains. The biofilm cell densities for other *Enterobacteriaceae* also varied considerably. *Esch. coli* K12 and *Ent. cloacae* formed lower biofilm cell densities than other *Enterobacteriaceae*, and *A. calcoaceticus* produced negligible biofilm; *ca.* 10 cfu/cm. The reason for the variation is unknown, but may be linked to cell surface hydrophobicity as well as surface structure. Other attachment factors warrant further investigation such as curli fimbriae and cellulose production. These have been reported in *Cronobacter* spp. *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. (Lehner et al. 2005; Zogaj et al. 2003).

Apart from *Ent. cloacae*, the bacterial biofilms which formed on silver-impregnated tubing were not significantly less than on PVC and PU tubing. The reason for the susceptibility of *Ent. cloacae* compared with other *Enterobacteriaceae* to silver ions is unknown. Nevertheless, overall the results demonstrate a lack of antibacterial activity by the silver ions when impregnated in the plastic. This could have been due to the presence of a conditioning film of formula components acting as a barrier to the diffusion of the silver ions. Consequently, silver-impregnated tubing does not appear to be a means of preventing bacterial biofilm formation on enteral feeding tubes.

Laboratory studies demonstrated the attachment of *Cr. sakazakii* to enteral feeding tubes within 2 h of exposure (Fig. 2). In these experiments, the tubes were flushed every 2 h to mimic hospital practices. The biofilm subsequently inoculated the fresh infant formula in the lumen of the tube and the bacteria grew to ~10^8 cfu/ml. If this occurs *in vivo* then the lumen bacteria will be flushed into the neonate's stomach with each subsequent feed. Electron microscopy of enteral tubes incubated with *Cr. sakazakii* ATCC 12868 showed clumps of cells on the surface and within precipitated feed matrix (Fig. 2).

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This study demonstrates that *Enterobacteriaceae* and *Acinetobacter* gensp. 13 are able to attach and grow on enteral feeding tubes as bacterial biofilms. It is plausible that this biofilm will both inoculate subsequent routine feeds and as the biofilm ages, clumps of cells will be shed which may survive passage through the neonate's stomach due to protection from the acidity. Since low birth weight neonates have a low immune status and lack a competing intestinal bacterial flora, these biofilm-associated organisms could result in neonatal infections (Townsend & Forsythe, 2008). Therefore bacterial growth on enteral feeding tubes will constitute a risk factor to neonatal health. This issue warrants further investigation to reduce neonatal exposure to opportunistic pathogens, in particular susceptible neonates receiving enteral feeding due to possible bacterial growth *in situ*.

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