Institution: Nottingham Trent University

Unit of Assessment: A03: Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Safer feeds for babies: international recognition and detection of *Cronobacter* spp. as an emergent bacterial pathogen associated with neonatal meningitis

1. Summary of the impact (indicative maximum 100 words)

Microbiology research at NTU has helped worldwide to lower the risk of severe infections among newborn babies from consuming bacterially contaminated powdered infant formula. The work addressed widespread public concern over the emerging dangers of the bacterial pathogen *Cronobacter* spp. Our research findings have informed and facilitated improvements in methods for *Cronobacter* spp detection and the understanding of neonatal exposure routes and risk factors. In turn, this knowledge has contributed to safer production of the formula itself, changes in international legislation and regulations and, from 2008, the implementation of new World Health Organisation infant formula preparation guides.

2. Underpinning research (indicative maximum 500 words)

Since 2003, Steve Forsythe (Professor of Microbiology since 2008) and colleagues (Drs G. Manning, M. Loughlin, S. Townsend and J. Caubilla-Barron) have led research into the then newly-emergent bacterial pathogen *Cronobacter* spp. (formerly known as *Enterobacter sakazakii*). They have published 46 peer reviewed papers on the organism, 33 of which since January 2008.

The '*International Commission for Microbiological Criteria for the Safety of Foods*' (2002) had recognised the bacterium as an agent of concern for immune-compromised individuals, including an association with severe, life-threatening infections of newborn infants. Linking cases of infection in neonatal intensive care units with the recovery of the bacterium from powdered infant formula caused additional concern.

Initial studies (2003-2005) on the general nature of the organism revealed its ubiquity in foods, its rate of growth in reconstituted infant formula and its ability to adhere to inert surfaces. Iversen & Forsythe (Ref 1) published the first risk evaluation of the bacterium before the international regulatory community responded to international concerns (Food and Health Organization/World Health Organization [FAO-WHO] 2004). Additionally the then approved detection protocols were inadequate (due to poor taxonomic designation of the organism) and this led to inadequate control measures. In collaboration with Oxoid ThermoFisher research was undertaken (Ref 2) which led to a new chromogenic agar for detecting the bacterium, the Druggan-Forsythe-Iversen chromogenic agar (DFI), and official recognition of the genus *Cronobacter* in place of *Enterobacter sakazakii*.

The second research phase (2005-2008) secured international approval and subsequent commercialisation of the DFI agar for the reliable and robust recovery of the bacterium from powdered infant formula. On the basis of his expertise, Professor Forsythe was the sole academic invited to participate at all FAO-WHO risk assessments meetings for the microbiological safety of powdered infant formula (2004, 2006, 2008). In response to the FAO-WHO (2008) call for data on the microbiological safety of follow-up formulas, Professor Forsythe organised a collaborative survey across 7 countries for *Cronobacter* spp. in follow-up formulas (intended age >6 months). These data were submitted to the FAO-WHO (2008) to evaluate risk, and published separately (Ref 3).

The third research phase (2009- to date) uses genotyping and whole genome studies with Professor M. McClelland (Vaccine Research Institute of San Diego, and University of California) to advance our knowledge of the diversity of the organism and support regulatory requirements. This is essential for method validation which requires the target organism to be precisely defined such that all species and strain variations are detectable. The formal acceptance of a new bacterial genus, *Cronobacter*, in place of *E. sakazakii*, enables industry and regulators to detect and control

the organism in powdered infant formula. A multilocus sequencing typing scheme (MLST) supported by an open access, curated database (www.pubMLST.org/cronobacter) was established in conjunction with Professor Chris Dawson (Warwick University) and hosted by Dr Keith Jolley (Oxford University) (Ref 4).

The genotyping and genomic analyses have

- Contributed to the definition of the new bacterial genus *Cronobacter,* and recognition of two new *Cronobacter* species.
- Guided whole genome sequencing projects across the genus (Ref 5).
- Extended our knowledge of the diversity of *Cronobacter*, whilst also confirming the reliability of the recovery methods during phase 2 of the research (Ref 6).
- Identified a clonal lineage as causing the majority of neonatal meningitis cases; 30 year retrospective study and highly publicised USA cases in 2011 (Hariri et al 2013, *Emerging Infectious Diseases*. 19:175-177. DOI.org/10.3201/eid1901.120649).

3. References to the research (indicative maximum of six references)

Citations and impact factors below refer to information according to http://wok.mimas.ac.uk on 18th October 2013.

1. Iversen, C. & Forsythe, S.J. (2003). Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. *Trends in Food Science and Technology*. 11: 443-454. DOI:10.1016/S0924-2244(03)00155-9. Impact Factor 4.135, Citations:190.
2. Iversen, C., Druggan, P. & Forsythe, S.J. (2004). A selective differential medium for *Enterobacter sakazakii*. *Intl. J. Food Microbiol.* 96: 133-139. DOI 10.1016/j.ijfoodmicro.2004.01.024. Impact Factor 4.135, Citations: 94.
3. Chap, J., Jackson, P., Siqueira, R., Gaspar, N., Quintas, C., Park, J., Osaili, T., Shaker, R., Jaradat, Z., Hartantyo, S.H.P., Abdullah Sani, N., Estuningsih, S., & Forsythe, S.J. (2009). International survey of *Cronobacter sakazakii* and other *Cronobacter* spp. in follow up formulas and infant foods. *Intl J Food Microbiol*.136:185-188. DOI:10.1016/j.ijfoodmicro.2009.08.005. Impact Factor 3.425, Citations: 26.
4. Baldwin, A., Loughlin, M., Caubilla-Barron, J., Kucerova, E., Manning, G., Dowson, C. & Forsythe, S. (2009). Multilocus sequence typing of *Cronobacter sakazakii* and *Cronobacter malonaticus* reveals stable clonal structures with clinical significance which do not correlate with biotypes. *BMC Microbiology* 9:223. DOI: 10.1186/1471-2180-9-223. Impact Factor 3.104, Citations: 29.
5. Kucerova, E., Clifton, S.W, Xia, X-Q, et al. (2010). Genome sequence of *Cronobacter sakazakii* BAA-894 and comparative genomic hybridization analysis with other *Cronobacter* species. *PLoS ONE* 5(3): e9556. DOI:10.1371/journal.pone.0009556. Impact Factor 3.730, Citations: 48.
6. Joseph, S., Sonbol, H., Hariri, S., Desai, P., McClelland, M., & Forsythe, S.J. (2012). Diversity of the *Cronobacter* genus as revealed by multi locus sequence typing. *J Clin. Microbiol*. 50:3031-3039. DOI:10.1128/JCM.00905-12. Impact Factor 4.068, Citations: 16.

4. Details of the impact (indicative maximum 750 words)

Research findings and advice from Professor Forsythe's team have led to (1) revisions in international regulations on the safe feeding of infants in hospitals and in the home, and (2) improvements in the microbiological safety of manufactured powdered infant formula.

1. Influence on international regulatory affairs

1a. Three FAO-WHO **risk assessments** on the microbial safety of powdered infant formula (2004, 2006, 2008, source of corroboration 1a) were informed by the Unit's research on bacterial pathogens in infant formula and advice from Professor Forsythe as an expert at the risk assessment workshops. The reports made recommendations to worldwide regulatory authorities; see 1c below.

1b. WHO **risk communication** guidelines on the hygienic preparation of powdered infant formula were revised in 2007 (source of corroboration 1b) and were used to inform individual governmental regulatory authorities; see 1c below.

1c. The recommendations from 1a & b above were adopted (2008-) by the worldwide regulatory community (the **risk managers** in, for example, Canada, Germany, New Zealand, United Kingdom, United States) regarding the safe preparation of formula in hospitals and homes (sources of corroboration 2a, b, c).

1d. The development of an online **risk assessment** model by the Joint FAO-WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) http://www.mramodels.org/esak was based on the FAO/WHO reports (1a above) for use by formula manufacturers and regulatory bodies; still in current use (source of corroboration 1c).

1e. Prior to 2008 there was no international requirement for the detection of *Cronobacter* in powdered infant formula. The risk assessments of FAO-WHO (1a above) informed by the research in this Unit led to changes in **risk management** by the Codex Alimentarius Commission with the introduction of international microbial criteria (<1 *Cronobacter* cell/10g powdered infant formula) for commercially produced powdered infant formula (CAC 2008). These new criteria are now implemented into international legislation by respective regulatory bodies. As explained below, compliance with these criteria required the development of reliable detection methodologies, based on an accurate definition and understanding of the *Cronobacter* genus (source of corroboration 3).

***2. Cronobacter* genus recognition and detection methodology implementation**

A consequence of the FAO-WHO risk assessments was the new international legal requirement (Codex Alimentarius Commission) for the absence of *Cronobacter* spp. in powdered infant formula (test weight 300g). Therefore robust and reliable detection methods were needed to ensure both consumer protection (presence of false negative results - a potential health hazard) and manufacturer protection (presence of either false negative [brand protection and legislative compliance] or false positive results [costly batch rejection]). In response to this need, Professor Forsythe, in collaboration with Oxoid ThermoFisher, co-developed the selective DFI chromogenic agar for *Cronobacter* spp (Ref 2). The DFI agar was used in the international survey for the specific isolation of *Cronobacter* spp. from follow up formula co-ordinated by Professor Forsythe and survey results were used in the FAO-WHO (2008) report and separately published (Ref 3). This agar is now:

- Commercially available from international microbiological media manufacturers (eg. CM1055 Oxoid, CSA-DFI LabM, Chromocult Merck)
- Used by powdered infant formula manufacturers
- Compliant with International Standards Organization (ISO) standard ISO/TS 22964
- A Food and Drug Administration (USA) recommended method (FDA 2012).

Sources of corroboration 4a and 4b.

3. Size of 'at risk' population (beneficiaries) and commercial interest

The scale of the exposure to risk can be approximated through the number of infant formula feedings. The world sales of infant formula are nearly a million tons per year (907000 tons in 2007 estimate by UBIC Consulting). Based on 200 feedings in a one-month period, ~250 babies/million population per year weighing 2000g or less (data source FAO-WHO 2004), and since infant formula is given for up to the first 3-6 months of life, the number of feeds/year/million population and therefore the size of the 'at risk' population is estimated to be 150000-300000/annum.

Uncertainties about quality of product have dramatic financial consequences for formula producers. For example, in December 2011 Mead Johnson's share prices fell by 11% due to product withdrawal by Wal-Mart Stores Inc. following a publicised baby's death from *Cronobacter* infection; source of corroboration 4c.

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. International regulatory agencies use of data generated by Professor Forsythe's team.

a. FAO-WHO recommendations are used by the Codex Alimentarius Commission (CAC) for harmonising international food standards, guidelines and codes of practice for consumer protection and fairness in the food trade. The Commission also promotes coordination of all food standards work undertaken by international governmental and non-governmental organizations.
Professor Forsythe co-authored the microbiological sections of the three FAO-WHO reports, the final one being: FAO-WHO (2008) Third Workshop on *Cronobacter* (*Enterobacter*) *sakazakii* in powdered follow up formula, Microbiological Risk Assessment Series No.15.
(http://www.who.int/foodsafety/micro/jemra/meetings/formula/en/index.html).

b. WHO (2007) Guidelines for the safe preparation, storage and handling of powdered infant formula which was used to inform individual governmental regulatory authorities listed below in 2. See Prof Forsythe's research cited in
http://www.who.int/foodsafety/publications/micro/pif2007/en/index.html.

c. JEMRA (Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment), which aims to make risk assessment tools more accessible and user-friendly to the wider food safety community, is linked to the Codex Alimentarius Commission and guides countries to adopt risk-based approaches. JEMRA used the data from Professor Forsythe's team in its risk model for infant formula. JEMRA web site for *Cronobacter* (http://www.mramodels.org/esak/); this guide has not been superseded.

2. Governmental regulatory authorities implementation of feeding practices guidance, worldwide (includes UK, USA, Canada, Germany and New Zealand) which have used Prof Forsythe's data.

Examples are:

a.UK:
http://www.nhs.uk/conditions/pregnancy-and-baby/pages/making-up-infant-formula.aspx#close
Note also that in January 2013 the UK Department of Health (Professor Dame Sally C Davies [Chief Medical officer] and Viv Bennett [Director for Public Health Nursing]) sent a letter to health professionals stating that water at 70^{o}C or above should be used to make up powdered feed in agreement with the WHO guidelines.

b. USA: http://www.cdc.gov/features/cronobacter/

c. Germany:
http://www.bfr.bund.de/cm/343/empfehlungen-zur-hygienischen-zubereitung-von-pulverfoermiger-saeuglingsnahrung.pdf

3. International standard methods approval for DFI agar usage:

a. International Standards Organisation (ISO) method for microbiological analysis of milk and milk products for *Cronobacter*; http://www.iso.org/iso/catalogue_detail.htm?csnumber=41258

b. Food & Drug Administration (2012) Bacteriological Analytical Manual. Chapter 29;
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm289378.htm

4. Commercialisation of DFI agar and infant formula product protection

The chromogenic DFI agar is compliant and approved by ISO and FDA standards, and testing requirements of Codex Alimentarius, for use by infant formula manufacturers (eg. Mead Johnson Nutraceuticals (USA) and Nestle) for the surveillance and control of *Cronobacter*.
Examples of DFI from current microbiological media manufacturers catalogues:

a. Oxoid Thermofisher:
http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM1055&org=65&c=UK&lang=EN

b. LabM: http://www.labm.com/product.asp?id=1582

Infant formula manufacturer's financial loss due to concerns about product safety:

c. Reuters:
http://www.reuters.com/article/2011/12/29/us-meadjohnson-idUSTRE7BL17R20111229