Institution: Cardiff University

Unit of Assessment: Biological Sciences (5)

Title of case study: Minimising bacterial contamination risks for Unilever through application of microbial genomics

Period when the underpinning research was undertaken: 2004 - 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Eshwar Mahenthiralingam	Professor	01/06/1999 – present
Laura Rushton	Research Associate	01/05/2020 – present
Rebecca Weiser	Research Associate	19/02/2018 – present
Thomas Connor	Professor	01/11/2012 – present
Andrew Weightman	Professor	01/01/1984 – present

Period when the claimed impact occurred: 2014 - 2020

Is this case study continued from a case study submitted in 2014? No

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

Many products such as cosmetics and toiletries are manufactured in non-sterile environments where microbial contamination can occur. Contamination can pose a serious health risk to consumers and lead to costly product recalls. A research programme between Cardiff University and global manufacturer, Unilever PLC, defined the risks of contamination by *Burkholderia* bacteria and other priority contaminants for the company. Based on Cardiff's data, Unilever accurately identified contaminants, developed new preservative compositions, and implemented robust monitoring and risk prevention strategies. This successfully reduced bacterial contaminations for the company and prevented product recalls, each of which cost at least £750K (and often millions when brand and manufacturer confidence is compromised).

2. Underpinning research (indicative maximum 500 words)

The *Burkholderia cepacia* complex (*Burkholderia*) encompasses a group of over 20 bacterial species, requiring specialist molecular approaches to accurately identify each strain. They are also highly antimicrobial-resistant bacteria, making them hard to treat in human infections. This resistance also makes them problematic contaminants as they are difficult to suppress through antimicrobial preservatives used in non-sterile industrial products. Unilever knew that unidentified *Burkholderia* species were a contamination risk and wished to understand if this risk could be eradicated, even though this is not required under global manufacturing regulations.

Cardiff University's team was in a unique position to work with Unilever on this issue as Mahenthiralingam had been the first to develop a single-gene approach to identification of *Burkholderia* in the early 2000s. This was subsequently developed by Mahenthiralingam into a multi-gene identification approach, multilocus sequence typing (MLST), enabling *Burkholderia* strains to be accurately identified and tracked on a global scale **[3.1]**. In the last five years, the group has also pioneered genome sequencing methods for high resolution tracking and risk analysis of *Burkholderia* bacteria. This unique expertise and MLST resource led Unilever to approach the Cardiff team and begin a joint research programme in 2004 totalling over £1M in award funding **[G3.1 - G3.5]**. This programme has provided pioneering data on bacterial contamination in non-sterile industrial products as follows:

2.1 Unilever asked the Cardiff team to accurately identify *Burkholderia* bacteria and understand contamination risk

The Cardiff team characterised bacterial strains from Unilever manufacturing incidents **[G3.1, G3.2]** and showed that:

- *Burkholderia* strains recovered from industrial products may be genetically identical to those encountered in clinical infections **[3.2]**, and thus have the genetic capacity to cause infections as a major risk factor.
- Multiple species of *Burkholderia* can cause industrial contamination **[3.3]**.
- Species and strains of *Burkholderia*, which pose a risk to vulnerable individuals, were encountered as contaminants at Unilever sites. For example, *Burkholderia cenocepacia* was the second most common species found in industrial contamination **[3.3]** and is also a species associated with global epidemics and rapid death in people with cystic fibrosis.
- Pumping preservatives out of cells (efflux) provided a key mechanism used by *Burkholderia* to survive within industrial products **[3.3]**.
- Accurate identification of the *Burkholderia* species and strain type were key to understanding the risks of infection associated with contamination.
- Testing the susceptibility and tolerance to preservatives of certain *Burkholderia* strains **[3.3]** could inform product preservation strategies to minimise repeat contamination.

2.2 Unilever subsequently asked Cardiff researchers to identify other priority contaminants and develop a single comprehensive genomic database of problematic bacterial strains

In 2011, Unilever commissioned the Cardiff team to investigate an additional priority contaminant, *Pseudomonas aeruginosa* (*P. aeruginosa*) **[G3.3, G3.4, G3.5]**. Findings from this research revealed that:

- Unilever's system for growing cultures to test products for *P. aeruginosa* was robust, enabling Unilever to maintain their standard protocols rather than upgrade to more expensive methods **[3.4]**.
- *P. aeruginosa* contamination strains had larger genomes than strains from other sources, and high-risk industrial strains could be rapidly identified by the presence of a large and unique plasmid biomarker **[3.5]**.

The research also identified that antimicrobial resistant *Enterobacteriaceae* are key contaminants of future concern for Unilever, resulting in the funding of a new program to characterise risk and prevention strategies to eliminate contamination by these bacteria **[G3.6]**.

The Cardiff team further showed that, between 2005 and 2018, 49% of microbial contaminants reported to the European Commission non-sterile food product recall database (Safety Gate) had been classified as unidentified **[3.6]**. Cardiff's genomic approach, however, provided Unilever with a single database of 269 new genome sequences of all their contamination incident bacteria (including *Pseudomonas*, *Burkholderia*, *Enterobacteriaceae* and other priority industrial strains), which enabled accurate identification and risk understanding. This database, and the joint research programme, were fundamental in informing Unilever's global strategy to reduce the risk of bacterial contamination in their manufacturing processes and maintain their leading position as a global manufacturer of non-sterile products (see Section 4).

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

[3.1] Baldwin A, **E. Mahenthiralingam**, K.M. Thickett, D. Honeybourne, M.C.J. Maiden, J.R. Govan, J.J. LiPuma, P. Vandamme and C.G. Downson (2005). Multilocus sequence typing scheme that provides both species and strain differentiation for the *Burkholderia cepacia* complex. *J Clin Microbiol* 43(9): 4665–4673 (DOI: 10.1128/JCM.43.9.4665-4673.2005)

[3.2] Baldwin, A., **E. Mahenthiralingam**, P. Drevinek, P. Vandamme, J. R. Govan, D. J. Waine, J. J. LiPuma, L. Chiarini, C. Dalmastri, D. A. Henry, D. P. Speert, D. Honeybourne, M. C. Maiden and C. G. Dowson (2007). Environmental *Burkholderia cepacia* complex isolates in human infections. *Emerg Infect Dis* 13(3): 458-461 (DOI: 10.3201/eid1303.060403)

[3.3] Rushton, L., A. Sass, A. Baldwin, C. G. Dowson, D. Donoghue and **E. Mahenthiralingam** (2013). Key role for efflux in the preservative susceptibility and adaptive resistance of *Burkholderia cepacia* complex bacteria. *Antimicrob Agents Chemother* 57(7): 2972-2980 (DOI: 10.1128/AAC.00140-13)

[3.4] Weiser, R., D. Donoghue, **A. Weightman** and **E. Mahenthiralingam** (2014). Evaluation of five selective media for the detection of *Pseudomonas aeruginosa* using a strain panel from clinical, environmental and industrial sources. *J Microbiol Methods* 99: 8-14 (DOI: 10.1016/j.mimet.2014.01.010)

[3.5] Weiser, R., A. Green, M. Bull, E. Cunningham-Oakes, K. Jolly, M. Maiden, A. Hall, C. Winstanley, **A. Weightman**, D. Donoghue, A. Amezquita, **T. Connor**, **E. Mahenthiralingam** (2019). Not all *Pseudomonas aeruginosa* are equal: strains from industrial sources possess uniquely large multireplicon genomes. *J Microb Genom* 5 (7) (DOI: 10.1099/mgen.0.000276)

[3.6] Cunningham-Oakes, E., **R. Weiser**, T. Pointon, **E. Mahenthiralingam** (2020). Understanding the challenges of non-food industrial product contamination. *FEMS Microbiol Lett* 366 (23) (DOI: 10.1093/femsle/fnaa010)

Selected grants:

[G3.1] Mahenthiralingam, E. *The molecular basis for preservative resistance in Burkholderia cepacia complex bacteria* (L. Thomas [now Rushton] CASE PhD), 2007 – 2011, BBSRC, KEBM101, £65,000

[G3.2] Mahenthiralingam, E., **Rushton, L**. *Development of bioluminescent and rapid methods for preservative efficacy testing*, 2011 – 2013, Technology Strategy Board, KTP8702, £202,141

[G3.3] Mahenthiralingam, E. *The resistance of Pseudomonas aeruginosato preservatives used in industrial formulations* (R. Weiser CASE PhD), 2011 – 2015, BBSRC, BB/F016557/1, £65,000

[G3.4] Mahenthiralingam, E. *Investigating the genetic basis of preservative resistance in an industrial Pseudomonas aeruginosastrain* (A. Green CASE PhD), 2013 – 2017, BBSRC, BB/L502078/1/1, £70,614

[G3.5] Mahenthiralingam, E. *The genomic basis of preservative resistance* (E. Cunningham-Oakes CASE PhD), 2016 – 2020, BBSRC, BB/M009122/1, £72,104

[G3.6] Rushton, L. *Characterising the diversity and antimicrobial resistance of Enterobacteriaceae bacteria as key risk industrial contaminants* (Ser Cymru II industrial fellowship), 2020 – 2022, Welsh Government, CU218, £124,366

Unilever funding contributions to these projects totalled an additional £347,087.

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

The outcomes from Cardiff and Unilever's joint research programme minimised the risk of bacterial contamination within Unilever's manufacturing processes on a global scale. Unilever's Beauty and Personal Homecare business has an annual turnover of €37.2BN, with consumers across the globe using their products **[5.1]**. The research resulted in safer products for consumers and substantial financial savings for the company. Impact was delivered as follows:

4.1 Providing accurate identification of bacteria contaminants and reducing costly contamination incidents

Unilever used Cardiff's research to set industry-leading standards in accurately identifying contaminants and managing the associated risks. The standards adopted by Unilever were considerably more robust than deemed necessary by regulatory compliance, since industry guidelines and European regulations do not require manufacturers to identify *Burkholderia* bacterial contaminations. As noted by Unilever's Peter Carew (R&D Director) and Stuart Campbell-Lee (Research Scientist) **[5.1, 5.2]**:

- *"Cardiff University researchers have assisted us in the development of a comprehensive database of 269 contaminant strain genomes, including 68* Burkholderia*"* **[5.2]**. This represents approximately a **17-fold increase** in Unilever's identified contaminant strains since 2016.
- *"The new methodology and accompanying genomic knowledge of microbial contaminant strains goes above and beyond regulatory requirements for preservation evaluation to ensure protection against* Burkholderia" **[5.2]**.

The research has been used by Unilever to:

- identify two *Burkholderia* contamination incidents in 2014 (a cosmetic product) and 2016 (a non-cosmetic product), which Unilever's commercial contract research provider (CRO) for microbial contamination was unable to identify **[5.1]**. The Cardiff team provided Unilever with accurate identification of these *Burkholderia* contaminants using MLST, genome sequencing, and resources not available to the CRO. Informed by Cardiff's expertise and high-resolution analysis, Unilever was able to address these incidents with targeted clean-up procedures **[5.1]**;
- implement robust strategies to combat bacterial contamination, based on Cardiff-derived data. Carew noted that: "*Since 2014 Unilever cosmetics have not endured a Burkholderia microbial contamination incident as a result of the actions taken from knowledge development achieved through this partnership, specifically improving factory hygiene practices and preservation system design"* **[5.1]**. This has prevented contamination and future product recalls. These normally cost in excess of £750K per contamination incident, as well as *"intangibles such as loss of future customer sales due to bad PR"* **[5.1]**.

4.2 Informing the development and patenting of new preservative compositions

Manufacturers face new challenges as consumer and regulatory demands move towards products with milder, more natural preservatives or preservative-free formulations. These are, however, at greater risk of microbial contamination **[5.1]**. Cardiff research provided Unilever with an understanding of antimicrobial susceptibility and synergy testing methods (a chequerboard assay) which led to the company developing and patenting three new preservative compositions for their products (2019 international patents: WO 2019/233753 A1, WO 2019/233757 A1, and WO 2019/233752 A1) **[5.2]**. These preservative compositions are designed to guard against contamination (based on Cardiff research **[3.3, 3.5]**), and will be used in a wide range of Unilever's personal care and hair care products, with a global market value of over US$10BN. As Unilever stated **[5.2]**:

- *"A method transfer was performed introducing synergy assessments using a 'chequerboard assay' for preservatives during Dr Laura Rushton's (Cardiff University) Knowledge Transfer Partnership (KTP) project*."
- *"This methodology and the panels of well characterised, genetically typed contamination strains have since become part of a valuable method to Unilever in order to establish synergistic preservative combinations as part of intellectual property filings impacting at an international level in 2019."*
- *"The use of these proprietary preservative combinations in a range of Unilever consumer products will ensure business continuity is maintained in this multi-billion Euro business sector."*

4.3 Informing risk prevention and Unilever's global research priorities

From April 2016, Unilever developed an internal research strategy entitled 'Genomic Preservation Science' from April 2016 **[5.2]**. Prior to Cardiff's research, Unilever was using conventional bacterial assays to identify bacteria, rather than advanced genomic techniques. This new strategy outlined Unilever's commitment to move away from conventional assays to adopt a robust genomic approach, aligned to benefits demonstrated by Cardiff research **[3.2, 3.3, 3.5, 3.6]**.

This strategy resulted in a closed industry-academia workshop to define Unilever's long-term capability roadmap (held in April 2017). The Cardiff team acted as academic consultants for Unilever, informing development of their strategy and advising on the invited academic delegate list, which included research leaders from Liverpool, Southampton and Cardiff Universities **[5.3]**. Unilever's genomic strategy for identifying, monitoring and eradicating potentially harmful bacteria has now been rolled out across Unilever's major research and development centres, within the UK, the United States, China and India **[5.2]**.

Cardiff's research enabled Unilever to: accurately identify contaminants and essentially stop *Burkholderia* contamination incidents occurring in their products; develop new preservative compositions to avoid bacterial incidents; and implement robust monitoring and a global genomic preservation research strategy designed to avoid costly product recalls. Campbell-Lee noted that the research has *"empowered the preservation team"* and is driving "*industry to a more prudent destination to ensure cosmetic and household goods are adequately preserved"* **[5.2]**.

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

[5.1] Testimonial: Peter Carew, R&D Director at Unilever PLC

[5.2] Testimonial: Stuart Campbell-Lee, Research Scientist at Unilever PLC

[5.3] Genomics Preservation Science Meeting Programme, 2017