Institution:
UNIVERSITY OF LIVERPOOL and LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Unit of Assessment:
UA01 – Clinical Medicine

Title of case study:
Diagnostic Tests for Cystic Fibrosis Epidemic Strains

1. Summary of the impact

Chronic lung infections due to *Pseudomonas aeruginosa* are the major cause of morbidity and mortality associated with cystic fibrosis. Some strains of *P. aeruginosa* transmit between patients (epidemic strains). The Winstanley group, at the University of Liverpool (UoL) since 1999, in collaboration with clinicians in Liverpool, has developed diagnostic PCR assays for identification of the most widely reported UK epidemic strains of *P. aeruginosa*. The NHS clinicians use these tests to make informed decisions about patient segregation leading to markedly reduced incidence of LES infections. Researchers internationally have adopted the UoL research results and strategy to tackle other transmissible strains and modify clinical procedures.

2. Underpinning research

Cystic Fibrosis (CF) affects over 9000 people in the UK alone, and CF patients require lifelong healthcare. An estimated £110m pa is spent on treating UK CF patients. Chronic lung infections due to *Pseudomonas aeruginosa* are the major cause of morbidity and mortality associated with CF. It is very important that CF patients are kept free from infection with *P. aeruginosa* for as long as possible because (i) once a chronic infection is established, it is never eradicated and (ii) the earlier the infection, the worse the patient prognosis. The UoL has played the main role in recognising the important contribution in CF of a limited number of epidemic (transmissible) strains of *P. aeruginosa*, in particular the Liverpool Epidemic Strain (LES), which is the most prevalent in the UK, and has also been reported in North America. Patients infected with the LES suffer greater morbidity than patients infected with other strains.

Research at the UoL (2002-2013) carried out by the Winstanley group (specialising in microbiology and infection) has included the design, development and publication of PCR assays to identify the LES and Midlands1 epidemic strains, and a multiplex PCR assay for the identification of the three most widely reported UK epidemic strains of *P. aeruginosa* (LES, Midlands1 and Manchester strain). The work involved collaboration with clinical colleagues, particularly Dr Martin Walshaw (Head of the Adult CF Unit, Liverpool and Honorary status in UoL). The clinical collaboration ensured the supply of relevant clinical samples and strains to enable testing of the assays, and facilitated the introduction into diagnostic laboratories, initially in Liverpool. The Winstanley group published a PCR assay for the LES in 2002 and further improvements to the assay in 2006, along with a PCR assay for another prominent epidemic strain (Midlands1). A multiplex PCR assays for three epidemic strains was published in 2008. Other publications by the Winstanley group were used to demonstrate the utility of the assays and generate the basic science used to design and improve the assays.

The assays were designed by using various genetic / genomic techniques to identify regions of the genomes of *P. aeruginosa* epidemic strains that could be targeted for the design of specific PCR assays (ie. regions present only in these strains).

Since being introduced into the routine analysis of strains from CF patients in Liverpool, these tests have been used to guide clinicians with respect to decisions regarding segregation of patients.

Craig Winstanley is Professor of Bacteriology in the UoL and has worked at the University continuously since 1999. There were various PhD students and postdoctoral researchers (under the supervision of Winstanley) involved in the work, as well as clinical collaborators (particularly Dr Martin Walshaw Head of the Adult CF Unit in Liverpool and honorary UoL academic), who were responsible for providing samples and clinical input. Dr Jo Fothergill (Research Fellow) also played a prominent role in the work (since 2005). Along with Winstanley, she was responsible for

developing the multiplex PCR assay.

3. References to the research

The impacts stem from a whole series of research papers published in peer-reviewed journals identifying, characterising and developing / refining diagnostic tests for epidemic strains, especially the LES. Some relevant publications are listed below.

1. **Mowat E**, **Paterson S**, **Fothergill JL**, **Wright EA, Ledson MJ, Walshaw MJ**, **Brockhurst MA** and **Winstanley C** (2011). Pseudomonas aeruginosa population diversity and turnover in cystic fibrosis chronic infections. American Journal of Respiratory and Critical Care Medicine 183:1674-1679. Citations: 39 Impact Factor: 11.041

2. **Winstanley C**, Langille MGI, **Fothergill JL**, Kukavica-Ibrulj I, Paradis-Bleau C, Sanschagrin F, Thompson NR, Winsor GL, Quail MA, Lennard N, Bignell A, Clarke L, Seeger K, Saunders D, Harris D, Parkhill J, Hancock REW, Brinkman FSL and Levesque RC (2009). Newly introduced genomic prophage islands are critical determinants of in vivo competitiveness in the Liverpool Epidemic Strain of *Pseudomonas aeruginosa*. Genome Research 19:12-23. Citations: 85 Impact Factor: 14.397

3. **Fothergill JL**, **Upton A**, Pitt TL, **Hart CA** and **Winstanley C** (2008). Diagnostic multiplex PCR assay for the identification of the Liverpool, Midlands 1 and Manchester epidemic strains of *Pseudomonas aeruginosa*. Journal of Cystic Fibrosis 7:258-261. Citations: 16 Impact Factor: 2.873

4. Salunkhe P, **Smart CH**, Morgan JAW, **Panagea S**, **Walshaw MJ**, **Hart CA**, Geffers R, Tümmler B and **Winstanley C** (2005). A cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* displays enhanced virulence and antimicrobial resistance. Journal of Bacteriology 187:4908-4920. Citations: 94 Impact Factor: 3.177

5. **Al-Aloul M**, **Crawley J**, **Winstanley C**, **Hart CA**, **Ledson MJ** and **Walshaw MJ** (2004). Increased morbidity associated with colonization by an epidemic *Pseudomonas aeruginosa* strain in cystic fibrosis patients. Thorax 59:334-336. Citations: 80 Impact Factor: 8.376

6. **Parsons YN**, **Panagea S**, **Smart CHM**, **Walshaw MJ**, **Hart CA** and **Winstanley C** (2002) Use of subtractive hybridization to identify a diagnostic probe for a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa*. Journal of Clinical Microbiology 40: 4607-4611. Citations: 34 Impact Factor: 4.068

7. **Fothergill JL**, White J, Foweraker JE, **Walshaw MJ**, Ledson MJ, Mahenthiralingam, E. and **Winstanley C** (2010). Impact of *Pseudomonas aeruginosa* genomic instability on the application of typing methods in chronic cystic fibrosis infections. Journal of Clinical Microbiology 48:2053-2059. Citations: 13 Impact Factor: 4.068

Grant awards relating to this work

2013-2016. **Cystic Fibrosis Trust**. Clinical Exploitation of Genomics Data Produced by the *Pseudomonas* International Consortium, £99,743, PI **C Winstanley**

2013-2016. **Cystic Fibrosis Canada**. Clinical Exploitation of Genomics Data Produced by the *Pseudomonas* International Consortium, C$316,000 (£204,000) CoI **C Winstanley**

2011-2014. **Wellcome Trust**. Genetic diversification and within-host evolution of chronic *Pseudomonas aeruginosa* infections in cystic fibrosis patients, £249,795, **S Paterson**, **C Winstanley** and **M Brockhurst**

2009-2012. **Wellcome Trust**. Understanding the role of temperate bacteriophages in the

ecology and evolution of *Pseudomonas aeruginosa* in the cystic fibrosis lung environment, £223,208 **C Winstanley** and **M Brockhurst**

2008-2011. **Dr. Hadwen Trust**. Response of populations of *P. aeruginosa* to antimicrobial challenge during chronic infections in CF, £134,954, **C Winstanley**

2008-2012. **NIHR**. Virulent epidemic strains of *Pseudomonas aeruginosa* in cystic fibrosis, NIHR, £153,762 (approx.) **C Winstanley** (A component of the Biomedical Research Centre in Microbial Diseases c.£20 million total grant)

2005-2008. **CF Trust/Big Lottery Fund**. Genetic factors contributing to the success of CF "superbugs" in the UK, £93,867, **C Winstanley** and **CA Hart**

2002-2005. **Cystic Fibrosis Trust**. Use of subtractive hybridisation to identify novel genomic regions of an epidemic strain of *Pseudomonas aeruginosa*, £62,705, **C Winstanley** and **CA Hart**

4. Details of the impact

Building on the work of the Winstanley group prior to this impact period, when the research led to wider recognition that transmissible strains of *P. aeruginosa*, and the LES in particular, are important in the context of CF, the group has achieved the following impacts:

1. The multiplex PCR test for transmissible strains (combining Winstanley group designed PCR tests for the LES and Midlands1 strains with a published PCR assay for the Manchester strain) has been used routinely in the NHS diagnostic laboratories serving both Adult (Liverpool Heart & Chest Hospital) and Children's (Alder Hey Hospital) CF Units from 2008. This therefore represents **a new clinical product**.

2. The clinical uptake of the multiplex PCR test has extended **from local NHS to national level**, as HPA Colindale used it as its main assay to test *P.aeruginosa* isolates from CF Units across the UK for the majority of the period Jan 2008 to July 2013. The PCR tests developed in Liverpool have helped in the recognition of the presence of the LES in other CF centres. HPA Colindale receives thousands of *P. aeruginosa* isolates each year from CF Units across the UK for strain typing. Although they have more recently switched to wider use of an alternative typing method (VNTR), the PCR assays are still used occasionally by Public Health England, Colindale [10]. The LES remains the most common clone of *P. aeruginosa* associated with CF infections in the UK (Martin et al. 2013 J Med Microbiol on-line ahead of print).

3. The use of the multiplex PCR test has allowed clinicians to make informed decisions about patients with transmissible strains of *P. aeruginosa* with respect to segregation of patients, because patients are cohorted for treatment depending on their microbiological status (ie. LES-positive or LES-negative). This has had an **impact on the health** of CF patients because the use of this test has contributed to the fact that new cases of LES infections are extremely rare in Liverpool. The main beneficiaries are CF patients who are not yet infected with *P. aeruginosa*. Prior to the use of PCR tests to identify patients infected with transmissible strains, there was no patient segregation. The consequence of this can be seen from the fact that prior to its discovery and the development of a simple test, the LES was already present in 80% of *P. aeruginosa*-infected patients in the Liverpool paediatric unit (Cheng et al 1996 Lancet 348**:**639-642, Panagea et al. 2004 Molecular Diagnosis 7:195-200). Most of these patients have now moved on to the adult unit. Because of effective segregation measures, there are now only small numbers of LES-infected patients at the Liverpool paediatric unit, and there have been no new cases for several years. Cases in the paediatric CF Unit in Liverpool have decreased from 47 LES-positive patients in 2003 to 8 LES-positive patients in 2009. In the adult unit there was a reduction in the proportion of patients with LES (2003 to 2009) from 71% to 53% [12,13]. Since 2009, there have been

no new cases of LES-infected patients in the Adult CF Unit other than patients new to the unit (ie. transferred from the paediatric centre or new to the region). There is a clear clinical benefit to restricting the spread of the LES, which is associated with increased patient morbidity (Al-Aloul et al. 2004 Thorax 59:334-336). This increased morbidity has been shown to be true also for patients in North America infected with the LES (Aaron et al. 2010 JAMA 304:2145-2153). It is important to note that the impact of segregation based on genotyping is ongoing. Patients, including new patients and first-time *P. aeruginosa* infected patients, are regularly monitored using the PCR tests, enabling LES-positive patients to be treated apart from non-LES, and minimising the threat of further spread of the strain. It is likely that there has also been impact at other CF centres. Public Health England has confirmed that "Professor Winstanley's research has played an important role in the significant extensions of lifespan and quality of life improvements experienced by CF patients in England in the last few years" [10].

4. In addition, the research highlights the existence of transmissible strains of *P. aeruginosa* (thought unlikely before Liverpool's initial LES report in 1996) **has impacted on researchers** worldwide and contributed to the discovery of other transmissible strains (Fothergill, Walshaw & Winstanley 2012 Eur Resp J 40:227-238), leading to changes in clinical procedures aimed at control of cross infection.

5. The use of our PCR assay is recommended in guidelines published by the UK Cystic Fibrosis Trust Microbiology Laboratory Standards Group designed to underpin the development of National Standard Operating procedures for the processing of respiratory samples submitted from people with Cystic Fibrosis [8]. This was circulated to all diagnostic laboratories in the UK serving CF units. Hence, the assay has impacted on **policy change**.

5. Sources to corroborate the impact

Each source listed below provides evidence for the corresponding numbered claim made in section 4 (details of the impact).

8. "Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis" (2010) https://www.cysticfibrosis.org.uk/media/82034/CD_Laboratory_Standards_Sep_10.pdf. Section 4.3.1 gives details of a multiplex-PCR test developed in Liverpool for three epidemic strains (LES, Midlands1 and Manchester), quoting two UoL studies [3,7] (**Fothergill** et al. 2008; **Fothergill** et al. 2010).

9. Contact: Alder Hey Children's Hospital, CF paediatric unit, can confirm that the PCR tests have been used routinely to screen CF patients in Liverpool and corroborate the clinical impact in terms of reduced incidence of LES infection.

10. Letter: Public Health England, Colindale.

11. Contact: Papworth, can confirm that the PCR targets devised by us for the LES (PS21 and LES-F9) are used in PCR assays.

12. The publication Ashish et al. 2013 Journal of the Royal Society of Medicine 4:1 includes data supporting the clinical impact in terms of reduced incidence of LES infection in Liverpool.

13. Morris D, Fallon L, Heaf L, Burrows EF, Wallace H, McNamara PS, Winstanley C, Southern KW. (2009) A reducing prevalence of the Liverpool Epidemic Strain of Pseudomonas aeruginosa in children attending the index paediatric clinic. Ped Pulmonol vol 32 pp 325