Institution: St George's, University of London

Unit of Assessment: 1 Clinical Medicine

Title of case study: Novel molecular serotyping technology advances worldwide pneumococcal vaccine impact and development

Period when the underpinning research was undertaken: 2007 to 2015

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:
Dr Jason Hinds	Reader in Translational Pathogen Genomics, Senior Lecturer, Research Scientist	2017 – 2020 (present) 2015 – 2017 1999 – 2015
Dr Katherine Gould	Laboratory Manager, Postdoctoral Research Assistant	2005 – 2020 (present) 2001 – 2005
Dr Adam Witney	Reader in Bioinformatics Bioinformatics Scientist, Biochemist	2018 – 2020 (present) 2015 – 2018 2001 – 2009
Prof Philip Butcher	Professor of Molecular Medical Microbiology	1989 – 2020 (present)

Period when the claimed impact occurred: 1 Aug 2013 to 31 July 2020

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

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

A novel genomics-based approach for molecular serotyping by microarray was developed for use in pneumococcal vaccine studies. This DNA microarray was the leading method in an international evaluation project, demonstrating high sensitivity and specificity, especially for detection of multiple serotype carriage. International roll-out of the technology to regional hubs in Australia and South Africa was facilitated by creation of a not-for-profit company to deliver associated services, products and software. The methodology has been widely adopted to analyse over 40,000 samples from vaccine studies in 25 countries and proven essential in monitoring the effectiveness of pneumococcal vaccination and guiding future vaccine development.

2. Underpinning research (indicative maximum 500 words)

Streptococcus pneumoniae is a major cause of death in children under five worldwide due to pneumonia, septicaemia and meningitis. Whilst nasopharyngeal colonisation with the pneumococcus is common in healthy children, this asymptomatic carriage is a precursor to disease. Study of pneumococcal carriage is therefore important with regard to transmission and disease, offering a more practical endpoint than disease for vaccine impact studies, especially in resource poor settings.

Pneumococcal conjugate vaccines (PCVs) are effective at both protecting against disease and reducing carriage of the 10 or 13 serotypes contained within the current vaccines. Identifying serotype prevalence enables assessment of pre-vaccine coverage plus monitoring of post-vaccine impact and possible serotype replacement by non-vaccine serotypes. Detection of multiple pneumococcal serotype carriage is essential to accurately determine circulating pneumococcal populations.

Developing a microarray to determine pneumococcal serotypes

The initial research was facilitated by GBP2,500,000 of Wellcome Trust funding of the Bacterial Microarray Group at St George's (BμG@S). Established as a Centre of Excellence for bacterial microarrays, BμG@S collaborated with Dr Stephen Bentley at the Wellcome Trust Sanger Institute and Dr David Aanensen at Imperial College following completion of a WHO-funded project to determine the genetic basis of all 90 known pneumococcal serotypes (Bentley et al., (2006) PLoS Genet. 2(3):e31). The aim of the BμG@S-led research collaboration was to translate this genetic knowledge into a genomics-based diagnostic tool, by designing a versatile molecular serotyping assay, able to detect multiple serotype carriage and to offer an enhanced endpoint for assessing pneumococcal vaccine studies.

The BμG@S molecular serotyping microarray was designed to detect the presence and relative amounts of pneumococcal capsule (*cps*) genes in genomic DNA extracts from clinical samples to identify serotypes and their relative abundance. This genomics-based approach also enabled additional simultaneous analyses, including detection of antimicrobial resistance determinants, co-colonising pathogens and related Streptococcal species plus genotyping by arrayCGH analysis of the pneumococcal genome.

Evaluating the effectiveness of the microarray

Detection of multiple serotype carriage was a key benefit of the microarray approach and shown to be significantly better than the current WHO recommended protocol in a study in Thailand [1]. Initial studies also demonstrated further advantages of the microarray to reveal new insights into non-typeable or mistyped samples analysed by other methods [2] and non-typeable isolates that had caused invasive disease in the USA [3]. The utility of the microarray for detection of multiple serotype carriage was again demonstrated in early pre-vaccine studies in Nepal [4] and Malawi [5].

Independent confirmation of the excellent performance and superiority of the molecular serotyping microarray was provided by a methods evaluation funded by the Bill & Melinda Gates Foundation. The PneuCarriage project systematically evaluated 20 different serotyping methods currently in use in laboratories worldwide, focusing especially on multiple serotype detection. The BμG@S molecular serotyping microarray was shown to be the leading method with very high sensitivity and specificity for detection of multiple serotype carriage [6]. An overall sensitivity of 99% for spiked samples and 95.8% for field samples was demonstrated. Moreover, the microarray determined accurately the relative abundance of serotypes. This study has led to adoption of the BμG@S molecular serotyping microarray in pneumococcal vaccine studies worldwide.

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

1. Turner P., Hinds J., Turner C., Jankhot A., Gould K., Bentley S.D., Nosten F. and Goldblatt D. (2011) Improved detection of nasopharyngeal cocolonization by multiple pneumococcal serotypes by use of latex agglutination or molecular serotyping by microarray. J Clin Microbiol. 49(5):1784-9. DOI: 10.1128/JCM.00157-11. Journal article cited 100 times (WOS 12.02.2021).

2. Salter S.J., Hinds J., Gould K.A., Lambertsen L. Hanage W.P., Antonio M., Turner P., Hermans P.W., Bootsma H.J., O'Brien K.L. and Bentley S.D. (2012) Variation at the capsule locus, *cps*, of mistyped and non-typable *Streptococcus pneumoniae* isolates. Microbiology. 158(6):1560-9. DOI: 10.1099/mic.0.056580-0. Journal article cited 50 times (WOS 12.02.2021).

3. Scott J.R., Hinds J., Gould K.A., Millar E.V., Reid R., Santosham M., O'Brien K.L. and Hanage W.P. (2012) Nontypeable pneumococcal isolates among Navajo and White Mountain Apache communities: Are these really a cause of invasive disease? J Infect Dis. 206(1):73-80. DOI: 10.1093/infdis/jis307. Journal article cited 19 times (WOS 15.02.2021).

4. Kandasamy R., Gurung M., Thapa A., Ndimah S., Adhikari N., Murdoch D.R., Kelly D.F., Waldron D.E., Gould K.A., Thorson S., Shrestha S., Hinds J. and Pollard AJ. (2015) Multi-

serotype pneumococcal nasopharyngeal carriage prevalence in vaccine naïve Nepalese children, assessed using molecular serotyping. PLoS One 10(2):e0114286. DOI: 10.1371/journal.pone.0114286. Journal article cited 19 times (WOS 15.02.2021).

5. Kamng'ona A.W., Hinds J., Bar-Zeev N., Gould K.A., Chaguza C., Msefula C., Cornick J.E., Kulohoma B.W., Gray K., Bentley S.D., French N., Heyderman R.S. and Everett D.B. (2015) High multiple carriage and emergence of *Streptococcus pneumoniae* vaccine serotype variants in Malawian children. BMC Infect Dis. 15:234. DOI: 10.1186/s12879-015-0980-2. Journal article cited 32 times (WOS 15.02.2021).

6. Satzke C., Dunne E.M., Porter B.D., Klugman K.P., Mulholland E.K, and PneuCarriage project group. (2015) The PneuCarriage Project: a multi-centre comparative study to identify the best serotyping methods for examining pneumococcal carriage in vaccine evaluation studies. PLoS Med. 12(11):e1001903. DOI: 10.1371/journal.pmed.1001903. Journal article cited 50 times (WOS 15.02.2021).

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

Influencing policy recommendations leading to take-up and use of services
Early in development of the technology, initial conference presentations and publications reporting the BμG@S molecular serotyping microarray were cited in the Updated Recommendations from World Health Organisations Pneumococcal Carriage Group [A]. Listed under new serotyping methods, advantages of the microarray approach were highlighted in terms of the additional simultaneous analyses possible. Furthermore, the recommendations indicated the importance of methods to detect multiple serotype carriage and noted the ongoing PneuCarriage project methods evaluation, in which the BμG@S microarray was subsequently shown to be the leading method [B, C].

Global adoption of a new diagnostic technology
Following completion of the PneuCarriage evaluation, the Bill & Melinda Gates Foundation funded roll-out of the BμG@S microarray technology [D, E]. Regional hubs were established at the Murdoch Children's Research Institute (MCRI) in Melbourne, Australia and the Respiratory and Meningeal Pathogens Research Unit (RMPRU) in Johannesburg, South Africa. Laboratory personnel from each site visited the UK for training and BμG@S personnel visited each institute to coordinate laboratory and equipment setup and provide onsite training. Each regional hub continues to process samples for vaccine studies within their geographic regions as part of the Gates Global Access Strategy supported by BUGS Bioscience, resulting in dissemination and adoption of the method worldwide [C, E].

New spin-out company created, with viability established and revenue generated
BUGS Bioscience Ltd. was founded in 2014 following business angel investment through the UK Government's Seed Enterprise Investment Scheme (SEIS) [F]. This St George's spin-out company was established as a not-for-profit to align with global health partners, such as Gates, GAVI and PATH, that were funding many of the vaccine studies. In the 6 years since its foundation, total turnover has exceeded GBP2,500,000 and the company has directly employed 4 individuals and sub-contracted 3 St George's personnel as required. This sustainability model enables support for ongoing research activity and global roll-out plus investment in new products, services and software development.

Demonstrating and monitoring the effectiveness of pneumococcal vaccination
Over 40,000 samples from studies in 25 countries have been analysed by molecular serotyping microarray through collaborations and research contracts with leading international research groups, NGOs and pharmaceutical companies [C, G]. These include large studies funded by the Bill & Melinda Gates Foundation and GAVI to assess PCV impact in resource poor settings. Microarray results from recent studies in Fiji, Mongolia, Cambodia and Lao PDR indicated a decrease of 63%, 52%, 39% and 23% in vaccine type (VT) carriage and an associated increase of 28%, 55%, 23% and 11% in non-VT carriage, respectively [G]. Importantly, a decrease of

26% in invasive pneumococcal disease was estimated in Cambodia following PCV introduction, reflecting these changes in carriage [G]. The comprehensive detection of all serotypes, non-typeable pneumococci and multiple carriage allowed statistically well-powered assessment of serotype changes not possible by other approaches. These carriage results are essential to monitor vaccine impact, inform future roll-out and support vaccine advocacy.

New clinical interventions using vaccines have been trialled
Numerous clinical trials have used molecular serotyping microarray results as primary or secondary endpoints [C, H]. These include studies in Nepal, Vietnam and The Gambia to evaluate novel dosing schedules for current PCVs. An ongoing study in The Gambia has assessed maternal or newborn vaccination to provide protection before routine infant vaccination becomes effective. Trials of new vaccines, a trivalent pneumococcal protein vaccine (Sanofi Pasteur PPrV) and an inactivated whole cell vaccine (PATH-wSP), were undertaken as serotype-independent alternatives to PCVs. The microarray was selected to measure the carriage endpoint more effectively due to its sensitive detection of multiple serotypes and combination of additional analyses, such as related species or antimicrobial resistance. This single assay provides much richer and clinically more informative data than other methods available, helping evaluation of these novel schedules and vaccines.

Entrepreneurial activity for design and delivery of new products and services
BUGS Bioscience operates as supplier of the custom designed microarrays and provider of high-quality molecular serotyping services. This integrated technology platform incorporates Senti-NET [I], a cloud-based software accessed via the web. Senti-NET was developed to support laboratory workflow, data analysis and sharing of results from the database of worldwide studies. The pneumococcal molecular serotyping microarrays were branded as Senti-SP along with a growing product portfolio of Senti-GBS, Senti-HI and Senti-NM microarrays developed by BUGS Bioscience for similar applications in Group B Streptococcus (*Streptococcus agalactiae*), *Haemophilus influenzae* and *Neisseria meningitidis* respectively [I]. The WHO listed these 4 key pathogens in the “Defeating Meningitis by 2030: A Global Road Map” [J] for which new diagnostics and surveillance activity will be required alongside vaccine roll-out and development. This presents future opportunities for wider adoption of the methodology to assess vaccine effectiveness, accelerate new vaccine development and monitor antimicrobial resistance for these major human diseases [C, J].

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

A. Standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. https://doi.org/10.1016/j.vaccine.2013.08.062
See Table 3 p173 for advantages of the microarray method (Ref 3 in table legend), Section 13 p174 for importance of multiple serotype carriage and PneuCarriage project. Citations in Refs 53, 55, 87, 88, 104, 105, 128, 130.

B. The PneuCarriage Project: a multi-centre comparative study to identify the best serotyping methods for examining pneumococcal carriage in vaccine evaluation studies.
https://doi.org/10.1371/journal.pmed.1001903
The BµG@S microarray methodology (Method 4 in Table 3 p7) is identified as the top-performing method in the Abstract p2 as indicated in Fig 1 p14, Fig 3 p18, Fig 4 p19 and Discussion para 3 p20. Citations in Refs 13, 41, 72 and 73.

C. Testimonial from Prof. Kim Mullholland, London School of Hygiene & Tropical Medicine and Murdoch Children’s Research Institute [pdf].
Supports the case for key advantages of the methodology, roll-out to South Africa and Australia and global adoption of the microarray technology in studies and clinical trials worldwide.

D. Bill & Melinda Gates Foundation: Roll-out to Australia and South Africa
https://www.gatesfoundation.org/about/committed-grants/2013/11/opp1084341

E. Testimonial from Dr. Gail Rogers, Bill & Melinda Gates Foundation [pdf]
Supports the case for widespread adoption of the leading microarray method through delivery of the Global Access Strategy by St. George's, University of London and BUGS Bioscience.

F. BUGS Bioscience Ltd. registration at Companies House:
https://beta.companieshouse.gov.uk/company/08911333

G. Principal Investigators of studies worldwide using microarray technology [pdf]
Publications with hyperlinked DOIs are listed where available as external supporting evidence of microarray adoption. Publications within the REF2021 impact period are indicated with 'REF' and key papers supporting carriage results in Section 4 for Cambodia, Fiji, Lao PDR, Mongolia, and reduction in invasive disease in Cambodia, are indicated with 'ICS-1' to 'ICS-4'.

H. Clinical trials adopting microarray to assess primary or secondary endpoint [pdf]
All the trials listed were either initiated between Aug 2013 and July 2020 and/or had outcomes published within the REF2021 eligibility timeframe. Individual clinical trials can be accessed through the hyperlinks provided.

I. BUGS Bioscience company website product information for Senti-SP, Senti-GBS, Senti-HI, Senti-NM microarrays and Senti-NET software
https://bugsbio.org/products; https://bugsbio.org/software

J. WHO Defeating Meningitis by 2030: A Global Roadmap:
https://www.who.int/publications/m/item/defeating-meningitis-by-2030-a-global-road-map
Executive Summary para3 p3 highlights the four key pathogens to tackle and para4 p3 indicates the requirement for improved diagnostics and surveillance to assess burden of disease and future vaccine impact.