Polyhydroxyalkanoate beads as a particulate vaccine against

*Streptococcus pneumoniae* and *Neisseria meningitidis*

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Microbiology

at Massey University, Manawatu, New Zealand.

Majela González Miró

2017

Main Supervisor: Professor Bernd Rehm

Co-supervisors: Dr Zoe Jordens, Dr Vicente Vérez-Bencomo
Abstract:

*Streptococcus pneumoniae* and *Neisseria meningitidis* are the major causes of pneumonia and meningitis, respectively, worldwide. Capsular polysaccharide-protein vaccines (conjugate vaccines) provide protection against these diseases but not protection against infections caused by serotypes and serogroups not included in these vaccines. Proteins have been increasingly considered as antigens for vaccine development due to their more structurally conserved composition when compared to capsular polysaccharides. Proteins subunit vaccines are safe and protective; however, they have limitations such as serotype-dependent immunity, and low immunogenicity of the proteins, requiring adjuvant to be included in these formulations or delivery systems that enhance the desired immune response. In addition, complex production procedures are required, increasing production costs and therefore market prices making these vaccines inaccessible for many people affected by these diseases. Recently, bacterial storage polymer inclusions have been developed as protein antigen carriers. Polyhydroxyalkanoate, in particular 3-polyhydroxybutyrate (PHB) inclusions have been successfully bioengineered to display antigens from pathogens like *Mycobacterium tuberculosis* and Hepatitis C virus. These particulate vaccine candidates elicited both a Th1 and Th2 immunity patterns combined with a protective immune response against *Mycobacterium bovis* in mice.

This thesis focuses on the study of polyhydroxybutyrate (PHB) beads properties as a carrier/delivery system engineered to display antigens from extracellular bacteria. The antigens Pneumococcal adhesin A, Pneumolysin (proteins) and 19F capsular polysaccharide (CPS) from *Streptococcus pneumoniae*, and Neisserial adhesin A, factor H binding protein (proteins) and serogroup C CPS from *Neisseria meningitidis* were displayed on the PHB bead surface. These antigenic proteins were produced as fusion
proteins on the PHB bead surface, while the CPS was covalently attached by chemical conjugation. Mice vaccinated with these PHB beads produced strong and antigen-specific antibody levels. In addition, splenocytes from the same mice generated both IL-17A and IFN-γ production.

The antibodies elicited against antigenic pneumococcal proteins were able to recognise the same protein in the context of an *Streptococcus pneumoniae* whole cell lysate from more than six different strains, while antibodies produced after vaccination with 19F CPS conjugate to PHB showed high opsonophagocytic titers against the homologous strain. In the case of *Neisseria meningitidis*, bactericidal antibodies were elicited in mice vaccinated with PHB beads displaying proteinaceous and CPS antigens.

Overall, this thesis shows that PHB as particulate vaccine candidate holds the promise of a broadly protective vaccine that can be produced cost-effectively for widespread application to prevent diseases caused by *Neisseria meningitidis* and *Streptococcus pneumoniae*. 
With eternal love, gratitude and in memory of my mother

(Mercedes Miró Alonso, 1945-1993)
Acknowledgements

It has been a great experience and opportunity to work under the guidance and support of my main supervisor Professor Bernd Rehm. After hearing his oral presentation in Vaccipharma 2012, about PHA beads and their potential biomedical applications, combined with our understanding of the necessity to improve commercial vaccines against *Neisseria meningitidis* and *Streptococcus pneumoniae*, the idea to explore this platform for this purpose was born. This idea became a PhD project giving to me the possibility to enter a new field called nanotechnology. For all of these, I would like to thank Professor Bernd Rehm. In addition, I would like to thank my co-supervisors Dr Zoe Jordens and Dr Vicente Vérez-Bencomo for their support during this journey, respecting all the time their experiences, opinions and criticisms.

I thank members and ex-members of Bernd Rehm team, especially, Shuxiong Chen, Patricia Rubio, Jason Lee, Jason Smith, Jinping du, Natalie Burn, Kathryn Grage, Natalie Parlane, Andy Hollings…I learned a lot from them. The cooperation and interaction between my coworkers and myself, but also the weekly seminar allowed me to increase my scientific knowledge and my oral presentation skills.

I would like to thank my co-workers in The Finlay Vaccine Institute, Havana, Cuba, for all their professional and spiritual support, especially, Dr Dagmar García, Laura Marta Nodas, Yanet Estrada, Aylin Amador, Mildrey Fariñas, Sandra Madariaga, Dr Caridad Zayas, Neissa García, Aniuska Garces, MsC Amarilys Pérez, Leandro Camejo, Yury Valdés, Darielys Santana, Danaydis Fonseca, Ubel González, Elizabeth González, Dr Reinaldo Acevedo, Dr Reinaldo Oliva, Maria Onelia, Dr Barbara Cedré, Marilé García, MS Tamara Hernández, Alex Quintero Pérez, Dr Reinaldo Oliva Hernández, Dr Yanely Tirado, Rosmira Nicado.
I would like to thank Barry Bunn and Debra Cresswell for their support with English grammar. In addition, I would like to thank Jordan Taylor, Niki Minard and Matthew Savoian from the Manawatu Microscopy Imaging Centre for their assistance in TEM analysis. Prof Martin Hazelton and Dr Edgar Santos-Fernández are acknowledged for their assistance in statistical analysis of data in this thesis.

I am grateful for the scholarship support, by The MacDiarmid Institute for Advanced Materials and Nanotechnology and also, Massey University and The Finlay Institute for financial support.

I cannot forget to mention my friends from Cuba, New Zealand, Argentina, Mexico, Hungary, Colombia, Chile, Guatemala, Portugal, Spain, Germany, Austria, Italy, Ecuador, USA, France, The Netherlands, Paraguay, UK, Venezuela, Iran, India, Gana, Honduras thank you very much to all of them. But I would like to highlight my friend’s Dr Edgar Santos-Fernández, Dr Jimena Yapura, Dr Javier Flores and Tony Reid because you have helped a lot not only to be a better scientist but to be a better human being. Thank you very much.

Last but not least, I would like to thank my family especially my father (the love of my life and my example to follow) and my sister (my friend, my confidante….). Thank you forever. I will always love you both.
Preface

This thesis is written according to the regulations of the Handbook for Doctoral Study, revised in May 2016 by the Doctoral Research Committee. This thesis complies with the format of a thesis based on publication as described in the handbook.

Chapter 1

Introduction

This chapter was written by Majela González Miró as an introductory chapter for this thesis only and is not intended for publication.

Chapter 2

Self-assembled particulate PsaA as vaccine against Streptococcus pneumoniae infection

González-Miró, Majela¹², Rodríguez-Noda, Laura¹, Fariñas-Medina, Mildrey¹, García-Rivera, Dagmar¹, Vérez-Bencomo, Vicente¹, Rehm, Bernd H.A.²

Published: Heliyon 11 Apr 2017- Volume 3, Issue 4 Pharmaceutical Science, Biochemistry, Immunology

G.-Miró, M: Conceived and designed the experiments; performed and supervised the experiments; analysed and interpreted the data; Wrote the paper. R.-N. L., F-M. M: Performed the experiments. G.-R. D.: Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. V.-B. V: Contributed reagents, materials, analysis tools or data. R. B.H.A.: Conceived and designed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Chapter 3

Biologically assembled polyester beads displaying pneumolysin and capsular polysaccharide induce protective immunity against *Streptococcus pneumoniae*

Majela González-Miró¹,², Anna-Maria Radecker², Laura M Rodríguez-Noda¹, Mildrey Fariñas-Medina¹, Caridad Zayas-Vignier¹, Mabel Heránndez-Cedeño¹, Yohana Serrano¹, Félix Cardoso¹, Darielys Santana-Mederos¹, Dagmar García-Rivera¹, Yury Valdés-Balbín¹, Vicente Vérez-Bencomo¹, Bernd H.A. Rehm²,³


*Under review by Frontiers in Immunology, Jan 2018*

Chapter 4

Bioengineered polyester beads co-displaying protein and carbohydrate-based antigens enhance protective efficacy against bacterial infection

Majela González-Miró¹,², Laura M Rodríguez-Noda¹, Mildrey Fariñas-Medina¹, Barbara Cedré-Marrero¹, Sandra-Madariaga-Zarza¹, Caridad Zayas-Vignier¹, Mabel Hernández-Cedeño¹, Torsten Kleffmann³, Dagmar García-Rivera¹, Vicente Vérez-Bencomo¹, Bernd H. A. Rehm²,³

M. G.- Miró, L.M. R.-N., M. F.-M., B. C.-M., S.M.-Z., C. Z.-V., M. H.-C., T.K. performed the studies and analysed the data. D.G.-R., V.V.-B., M.G.- Miró, B.H.A.R. analysed and interpreted the data; contributed reagents, materials, analysis tools or data. M.G.- Miró, and B.H.A. R. conceived and designed and supervised the experiments. M.G.- Miró, and B.H.A. R. wrote the manuscript.

*Published: Scientific Reports Journal, 30 Jan 2018, Volume 8, Issue 1*
Chapter 5.

General Discussion, Conclusion and Future work

This chapter was written by Majela González Miró for this thesis only and is not intended for publication.
Table of Contents

Abstract: .................................................................................................................................i
Acknowledgements ................................................................................................................iv
Preface ......................................................................................................................................vi
List of Figures ..........................................................................................................................xiv
List of Tables ............................................................................................................................xvi
Abbreviations ..........................................................................................................................xvii
Chapter 1. Introduction ........................................................................................................1
1.1  *Streptococcus pneumoniae* ............................................................................................1
1.1.1 Pneumococcal diseases and epidemiology ................................................................. 1
1.1.2 Host immune defences against *Streptococcus pneumoniae* ........................................ 2
1.1.3 Relevant pneumococcal virulence factors .................................................................. 3
1.1.4 Pneumococcal vaccines ............................................................................................... 5
1.2  *Neisseria meningitidis* .....................................................................................................6
1.2.1 Meningococcal diseases and epidemiology ................................................................. 7
1.2.2 Host immune defences against *Neisseria meningitidis* ............................................... 8
1.2.3 Relevant meningococcal virulence factors .................................................................. 9
1.2.4 Meningococcal vaccines ............................................................................................. 11
1.3  Adjuvants and delivery systems .......................................................................................12
1.4  Polyhydroxyalkanoate beads as potential particulate vaccines ..................................... 13
1.5  General Hypothesis ..........................................................................................................18
1.6  Aims and scope of the thesis ......................................................................................... 18
1.7  References .........................................................................................................................19
Preface to the next Chapter .................................................................................................33
Chapter 2. Self-assembled particulate PsaA as vaccine against *Streptococcus pneumoniae* infection ................................................................................................................34
2.1  Abstract ............................................................................................................................35
2.2 Introduction .................................................................................................................................................. 36
2.3 Materials and methods .................................................................................................................................. 37
  2.3.1 Bacterial strains, oligonucleotides, plasmids and cultivation conditions ............................................. 37
  2.3.2 Construction of plasmids mediating production of PHB beads displaying PsaA .............................. 39
  2.3.3 Construction of the plasmid encoding N-terminally His-tagged PsaA for production of soluble PsaA .............................................................................................................................................. 39
  2.3.4 Production, isolation and purification of PHB beads ............................................................................ 39
  2.3.5 Production, isolation and purification of recombinant soluble protein ................................................... 39
  2.3.6 Confirmation of the PhaC in vivo activity using transmission electron microscopy (TEM) ................................................. .......................................................................................................................... 40
  2.3.7 Protein analysis ....................................................................................................................................... 40
  2.3.8 Measurement of the PHA bead size distribution and zeta potential .................................................... 40
  2.3.9 Analysis of immunological properties of PHB beads ........................................................................... 41
  2.3.10 Statistical analysis ............................................................................................................................... 43
2.4 Results .......................................................................................................................................................... 43
  2.4.1 Construction of plasmids mediating production of the PsaA-PhaC fusion protein and His6-PsaA .......................................................... .................................................................................................................. 43
  2.4.2 Production and characterization of PsaA displaying PHA beads ......................................................... 44
  2.4.3 Humoral immune response ................................................................................................................... 50
2.5 Discussion .................................................................................................................................................... 54
2.6 Acknowledgements ...................................................................................................................................... 57
2.7 References ................................................................................................................................................... 58
2.8 Supplementary material ............................................................................................................................ 63
Preface to the next Chapter ................................................................................................................................... 64

Chapter 3. Biologically assembled polyester beads displaying pneumolysin and capsular polysaccharide induce protective immunity against *Streptococcus pneumoniae* ..... 65
3.1 Abstract ....................................................................................................................................................... 66
3.2 Introduction ............................................................................................................................................... 67
3.3 Materials and methods ......................................................................................... 68
3.3.1 Strains and cultivation conditions ................................................................. 68
3.3.2 Construction of plasmids for production of soluble Ply and Ply displayed on PHB beads .................................................................................................................. 69
3.3.3 PHB bead production ...................................................................................... 69
3.3.4 Production of soluble Ply .............................................................................. 69
3.3.5 Conjugation of CPS to PHB beads ................................................................. 70
3.3.6 Transmission electron microscopy (TEM) ....................................................... 70
3.3.7 Proteins analysis by SDS-PAGE and immunoblot .......................................... 70
3.3.8 Immune response evaluation ....................................................................... 71
3.3.9 Statistical analysis ........................................................................................ 75
3.4 Results .............................................................................................................. 75
3.4.1 Bioengineering *E. coli* for production of Ply-PHB beads and soluble His6-Ply 75
3.4.2 Immunological properties of the various PHB beads and soluble proteins ...... 79
3.5 Discussion ........................................................................................................ 87
3.6 Acknowledgements .......................................................................................... 91
3.7 References ....................................................................................................... 93
3.8 Supplementary material .................................................................................. 100

Preface to the next Chapter .................................................................................. 101

Chapter 4. Bioengineered polyester beads co-displaying protein and carbohydrate-based antigens enhance protective efficacy against bacterial infection ......................... 102
4.1 Abstract .......................................................................................................... 103
4.2 Introduction ..................................................................................................... 104
4.3 Materials and Methods ................................................................................... 106
4.3.1 Ethics statement ......................................................................................... 106
4.3.2 Construction of plasmids mediating production of PHB beads ..................... 107
4.3.3 Construction of plasmids for production of soluble His-tagged proteins ....... 107
4.3.4 Production, isolation and purification of PHB beads and soluble proteins ...... 107
4.3.5 Conjugation of MenC polysaccharide (CPS) to carrier proteins.......................... 108
4.3.6 Transmission electron microscopy analysis (TEM)........................................ 108
4.3.7 Measurement of PHB bead size distribution and surface charge.................... 108
4.3.8 NMR spectroscopy .................................................................................. 108
4.3.9 Protein analysis ...................................................................................... 109
4.3.10 Protein and carbohydrate quantification..................................................... 109
4.3.11 Immunization schedule for proteinaceous antigens ...................................... 110
4.3.12 Immunization schedule for conjugated vaccine prototypes .......................... 110
4.3.13 Assessment of anti-NadA and anti-fHbp antibody titers in mice.................... 111
4.3.14 Assessment of anti-MenC antibody titers in mice ...................................... 111
4.3.15 Analysis of the Ig subclass profile in sera ................................................... 111
4.3.16 Analysis of the cytokine production .......................................................... 112
4.3.17 Serum bactericidal assay ......................................................................... 112
4.3.18 Statistical analysis .................................................................................. 113
4.4 Results ........................................................................................................ 114
4.4.1 Bioengineering of Escherichia coli for production of antigen-displaying PHB inclusions and soluble antigens................................................................. 114
4.4.2 Immunological properties of antigen displaying PHB beads versus soluble antigens......................................................................................................... 119
4.4.3 Chemical conjugation of capsular polysaccharides to PHB beads. ............... 120
4.4.4 Immunological properties of antigen-coated PHB beads displaying CPS ...... 123
4.5 Discussion ................................................................................................... 129
4.6 Acknowledgements ...................................................................................... 135
4.7 References ................................................................................................... 136
4.8 Supplementary material .............................................................................. 144
Chapter 5. General Discussion, Conclusion, and Future work .............................. 156
5.1 General Discussion ...................................................................................... 156
5.2 General Conclusion ...................................................................................... 160
5.3 Future work ........................................................................................................... 161
5.3.1 Gene design ....................................................................................................... 161
5.3.2 New targets ....................................................................................................... 161
5.3.3 Antigen Multivalency vaccine .......................................................................... 162
5.3.4 Mucosal Immunity ........................................................................................... 162
5.3.5 Immunological studies ...................................................................................... 162
5.4 References ............................................................................................................ 164
Appendix .................................................................................................................... 169
List of Figures

Chapter 1

Figure 1. Electron microscopy image of *Pseudomonas aeruginosa* containing PHA granules. ................................................................. 14

Figure 2. The biosynthetic pathway of PHB production ................................................................. 15

Figure 3. PHB granules Self-assembly model. .............................................................................. 16

Chapter 2

Figure 1. Schematic presentation of the construction of plasmid pET-14b-psaA-phaC encoding the PsaA-PhaC fusion protein for the formation of PHB beads in recombinant *ClearColi* ........................................................................... 44

Figure 2. TEM analysis of recombinant *ClearColi* cells (pMCS69) harbouring various plasmids and respective isolated PHB beads. ........................................................................... 45

Figure 3. SDS-PAGE and immunoblot analysis of proteins attached to PHB beads and purified His6-PsaA ........................................................................... 46

Figure 4. Immunological assessment of PsaA display on the PHB bead surface. ........ 48

Figure 5. Correlation between zeta potential and pH of various PHB beads ................... 50

Figure 6. Anti-PsaA IgG antibody response. ............................................................................... 51

Figure 7. Isotype IgG profile evaluated by direct ELISA using ELISA plates coated with 0.5 µg of soluble His6-PsaA. ........................................................................... 52

Figure 8. Recognition of PsaA in various serotypes of *S. pneumoniae* by sera from mice immunized with PsaA displayed on PHB beads or soluble PsaA ........................................................................... 53

Supplementary Figure 1. SDS-PAGE of whole cell lysates of the various *S. pneumoniae* serotypes ........................................................................... 63

Chapter 3

Figure 1. Schematic representation of genes encoding proteins relevant to this study .. 75

Figure 2. TEM images of *E. coli* with PHB inclusions and the corresponding purified PHB beads ........................................................................... 77

Figure 3. SDS-PAGE and immunoblot analysis of proteins attached to PHB beads as well as purified His6-Ply. ........................................................................... 78

Figure 4. Analysis of induction of anti-Ply antibodies by various vaccine formulations. ........................................................................... 80

Figure 5. IgG subclass profile as assessed by ELISA ................................................................... 81

Figure 6. Cytokine profiles induced by various vaccine formulations. .............................. 82
Figure 7. Cross-reactivity of anti-Ply antibodies with Ply from various serotypes of S. pneumoniae. .................................................................84

Figure 8. Schematic representation of conjugation reaction between activated serotype 19F polysaccharide and PhaC on PHB beads or soluble TT. ....................................................85

Figure 9. Analysis of anti-19F CPS antibody titers. .................................................................86

Figure 10. The opsonophagocytic activity of sera against S. pneumoniae serotype 19F. .........................................................................................................................87

Supplementary Figure 1. SDS-PAGE of whole cell lysates of the various S. pneumoniae serotypes..................................................................................................................100

Chapter 4

Figure 1. Biological production and characterization of antigen coated PHB beads...115

Figure 2. PHB bead production and their immunogenicity. ..............................................117

Figure 2. PHB bead production and their immunogenicity (continued). ................118

Figure 3. Schematic representation of the chemical conjugation of the CPS (MenC) to soluble and insoluble antigens displayed on PHB beads and characterization of their immunological properties.............................................................................................................121

Figure 4. Structural models of PhaC depicting lysine residues proposed as sites conjugated to the activated polysaccharide. ..................................................................................123

Figure 5. Immunogenicity studies of the various antigens conjugated to MenC.......125

Figure 6. Bactericidal activity of various sera.................................................................128

Supplementary Figure 1. FHbp confirmation of molecular identity and bead display by ELISA using a commercial monoclonal anti-fHbp antibody (JAR4, NIBCS, UK). ....144

Supplementary Figure 2. 1H NMR monodimensional spectra of CPS. .................145

Supplementary Figure 3. MenC confirmation of molecular identity by ELISA using a commercial anti-CPS (MenC) monoclonal antibody (NIBS, UK). .........................145

Supplementary Figure 4. Assessment of IgG subclass binding to MenC evaluated by ELISA. ..........................................................................................................................146

Supplementary Figure 5. Assessment of IgG subclass binding to NadA protein evaluated by ELISA. ..............................................................................................................147
List of Tables

Chapter 1
Table 1. Pneumococcal protein vaccines under study.................................6

Chapter 2
Table 1. Description of bacterial strains, plasmids and oligonucleotides used in this study ......................................................................................................................................................38
Table 2. Tryptic peptide fingerprinting analysis (MALDI-TOF/MS)..............47
Table 3. PHB bead yield and composition ......................................................49

Chapter 3
Table 1. Characteristics of plasmids and oligonucleotides used in this study......76
Table 2. Tryptic peptide fingerprinting analysis (Triple TOF) .....................79
Table 3. PHB beads yields and the antigen/ mg of wet beads ratio ................79

Chapter 4
Supplementary Table 1. Strains, Plasmids and primers ..............................148
Supplementary Table 2. Identification of fusion proteins by peptide fingerprinting analysis (MALDI-TOF/MS). ..............................................................149
Supplementary Table 3. Correlation between Zeta potential and pH of various PHB beads........................................................................................................149
Supplementary Table 4. Amount of neisserial antigen attached to PHB beads and immunization doses...............................................................150
Supplementary Table 5. Carbohydrate/protein ratios and carbohydrate yield after conjugation and purification .........................................................150
Supplementary Table 6. Conjugation site analysis results by liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS).........................151
Supplementary Table 7. IgG/IgM ratio after first (1D) and third (3D) blood collection assayed against MenC .................................................................155
Supplementary Table 8. Size distribution of PHB beads in vaccine formulations (µm) as measured by dynamic laser scattering ....................................155
Abbreviations

APCs: antigen presenting cells
APS: activate polysaccharide
BCA: Bicinchoninic acid assay
CLSM: Confocal Laser Scanning Microscope
CPS: capsular polysaccharide
CFU: colony-forming unit
CON A: Concanavalin A
DC: dendritic cell
DF: Dilution Factor
DIC: Differential interference contrast
DMEM: Dulbecco's Modified Eagle's Medium
DT: Diphtheria toxoid
ELISA: enzyme-linked immunosorbent assay
FCS: fetal calf serum
fHbp: factor H binding protein
GNA2091: genome Neisseria antigen 2091
GNA2091-fHbp-PhaC: genome Neisseria antigen 2091 fuse to factor H binding protein and PhaC
HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
Ig: Immunoglobulin
IgG: Immunoglobulin G
IgG1, IgG2a, IgG2b and IgG3: Immunoglobulin G 1,2a,2b,3
IgM: Immunoglobulin M
IL17A: cytokine 17A
INF-γ: Interferon gamma
LB: Luria-Bertani broth (Lennox)
M. bovis: Mycobacterium bovis
N. meningitidis: Neisseria meningitidis
S. pneumoniae: Streptococcus pneumoniae
E. coli: Escherichia coli
MALDI-TOF-MS/MS: matrix assisted laser desorption ionization-time of flight mass spectrometry
MW: molecular weight
NaBH₃CN: Sodium Cyanoborohydride
NadA: Neisseria adhesin A
NadA-PhaC: NadA-PhaC fusion protein
NIBSC: National Institute for Biological Standards and Control
OVA: Ovalbumin
OPA: opsonophagocytic assay
PBS: Phosphate Buffered Saline
PCR: polymerase chain reactions
PHA: Polyhydroxyalkanoate
PhA: β- ketothiolase
PhaC: Polyhydroxyalkanoate synthase
PhB: Acetoacetyl-CoA reductase
PHB: Polyhydroxybutyrate
Ply: Pneumolysin
Ply-PhaC: Pneumolysin fused to PhaC
PsA: Pneumococcal surface adhesin A
PsA-PhaC: Pneumococcal Surface protein A fused to PhaC
PsA: Pneumococcal Surface protein A
PspC: Pneumococcal Surface protein C
rpm: revolutions per minute
SBA: serum bactericidal activity
SD: standard deviation
SDS-PAGE: sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE)
SEM: standard error of the mean
TEM: Transmission electron microscopy.
Th17: Lymphocyte T helper 17
Triple TOF: mass spectrometry by Triple TOF
TT: Tetanus toxoid
TLR: Toll-like receptor
°C: degrees Celsius
¹H NMR: Proton nuclear magnetic resonance
UNICEF: United Nations Children's Fund
WHO: World Health Organization