Targeting Antimicrobial Resistance with Carbohydrate-Based Vaccines

Contents

Executive Summary

CarboMet is a four-year Collaborative Support Action ['CSA'] funded by the EC Horizon 2020 Future and Emerging Technologies initiative. The primary aim of the CSA is to mobilise the European academic and industrial community to identify generic measurement, data management and metrological challenges that must be met in order to advance and exploit carbohydrate knowledge and applications.

The potential for exploitation of carbohydrates lies in their diversity and structural complexity – subtle changes in the three-dimensional structure of a carbohydrate profoundly affects (for example) its ability to protect against or fight infectious disease. However, these subtle structural differences present a challenge for their analysis. Sophisticated measurement and metrological capabilities for analysing carbohydrates are available but are nowhere near as advanced or as routinely used in other areas such as gene sequencing.

Therefore as a first stage **CarboMet** has organised some open, Europe-wide workshops to identify key topics where our understanding needs to be advanced urgently, and where current limitations in our measurement, data management and metrological capabilities are hindering progress. The Workshops were also asked to recommend appropriate Work Programmes that should be supported by Horizon 2020 and its successor, Horizon Europe.

One of these workshops addressed the topic of the role of carbohydrate molecules in vaccine technology. This paper reports on the technological challenges that were identified at this workshop and lists a set of recommended Work Programmes which have been designed to help solve measurement, data management and metrological needs.

*This paper was formulated following discussions that took place at the following **CarboMet** workshops: "Polysaccharide Based Vaccines" Milan, Italy, 12th March 2018; "Polysaccharide Based Vaccines" Barcelona, Spain, 5th July 2017.

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Specific Challenge

The improvident use of antibiotics in clinical settings and animal husbandry has been a major cause for the rapidly developing problem of antimicrobial resistance (AMR), which is posing an increasing healthcare challenge for both developed and developing countries. The number of deaths due to infections resistant to antibiotics is projected to reach up to 10 million deaths annually by 2050 – more than cancer.[i] Alternative effective treatment strategies to antibiotics and preventative measures are urgently needed[ii].

Active and **passive vaccinations** (*see Box 1 for definitions*) offer highly promising alternative solutions for the treatment of infectious diseases that are resistant to antibiotics and have a number of advantages over small molecule antibiotics:
(i) Vaccines can be developed through semi-rational approaches once antigens have been identified;
(ii) Vaccines can be used to prevent infection (active) or to treat infection (passive);
(iii) Resistance is less of an issue with vaccination than with small molecule antibiotics;
(iv) Vaccines are very specific for target organisms with limited side-effects;
(v) Vaccines offer attractive opportunities to European Pharma Companies for novel biopharmaceutical products, which are already world-leading in this market.

Box 1:

*'A **vaccine** is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles antigenic **proteins** or **carbohydrates** of disease-causing microorganisms.*

***Active vaccines** stimulate the body's immune system to recognize the agent as foreign, produce antibodies and destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.*

***Passive vaccines** consist of externally administered antibodies against the pathogen.*

The carbohydrates at the cell surfaces of pathogens are particularly attractive candidates for vaccines[iii] and have been used successfully in polysaccharide and glycoconjugate vaccines[iv] such as the product Prevnar. Prevnar is a vaccine against pneumonia used to fight infections caused by pneumococcal bacteria, and achieved annual sales of approximately $6 billion in 2015[v]. These vaccines have established the principle of vaccine design based on microbial polysaccharides as a promising generic strategy.

Classical vaccine development has relied on using inactivated pathogen or fractions thereof. These are complicated biological mixtures with associated issues, such as safety considerations, around their production (i.e., large scale fermentation of hazardous pathogens), or quality control management. Recent advances in molecular biology and glycoscience provide us now with an opportunity to develop improved vaccines by addressing these issues around the carbohydrate component carbohydrate-based vaccines, importantly at the short timescales necessary to address increasing AMR. Whilst the fundamental technologies in Analysis, Synthesis, Target Development and Production have been solved, an integrated approach for their application in vaccine development is still missing and this lack of integration of technologies presents a bottleneck in the development of effective vaccines based on cell-surface polysaccharides.

Europe has great strengths in the area of glycoscience and related industries[vi] and **CarboMet** has brought together a group of international experts from academia and industry throughout Europe to identify the necessary next steps. To fully exploit the opportunities in fighting AMR using carbohydrate-based vaccines, **CarboMet** has identified the need for a cross-disciplinary programme focused on vaccine development against a list of pathogens that have been identified by the World Health Organisation (WHO) as a global priority[vii].

Scope of the Programme

Bacterial cell surface polysaccharides are crucial mediators for the host-pathogen recognition process in immunisation mechanisms[viii] and therefore represent key targets for vaccine design and innovation.

Two groups of microbial targets were identified as particularly relevant and promising for vaccine design using a 'One health' philosophy:

- For **human** health, targets were identified from a global priority pathogens list (Global PPL) produced in 2017 by the World Health Organisation (WHO) in response to the AMR crisis (*see Appendix for list*).
- Alongside these, it will be important to address the use of antibiotics in **veterinary** health within the same programme using common shared technologies in vaccine development against animal pathogens.

A number of work packages have been identified to address the challenges of carbohydrate-based vaccine development. These are described below.

WP1. INNOVATIVE FAST-SEQUENCING METROLOGY METHODS: The Molecular Identification of Bacterial Cell Surface Polysaccharide Targets

Highly targeted and effective carbohydrate-based vaccines can only be developed from a deep understanding of the molecular structure and conformation of the relevant cell surface polysaccharides.

The identification of suitable polysaccharide structures, at the fast timescale (days) required by the clinic, is currently a major bottleneck to rapid, accurate selection of the target structures, also ensuring coverage among different strains, in the development of carbohydrate-based vaccines. Whereas we now have fast sequencing methods for DNA and proteins, which have revolutionised modern medicine, carbohydrate sequencing is still very slow and requires highly specialised skills. Novel fast, automated and high-resolution *de novo* carbohydrate sequencing tools are urgently required that will give us access to high quality structure from easily accessible biological material within days.

Although this will be a major challenge, nevertheless it is envisaged that new combinations of (bio)chemical and (bio)physical methods and technologies must be developed, including more robust micro-extraction kits to isolate the carbohydrates of interest, identification of biosynthetic operons (e.g. for identifying glycosyltransferases) coupled with techniques such as gene knock-out technology, mass spectrometry, ion mobility[ix], IR and NMR spectroscopy. The metrology protocols so developed will require a central depository for acquired glycoanalysis data and readily available biological standards must also be developed. These could be naturally derived or synthesised (*see WP2 below*).

WP2. ACCURATE MONITORING FOR MANUFACTURING QUALITY CONTROL: Polysaccharide Production Methods

Once the molecular structures and conformations of appropriate polysaccharide targets have been identified at the small scale level, there is then a need for their production on an industrial scale for the manufacture of the novel vaccine. For this, there are a number of preparation approaches that can be used:-

- Isolation of naturally occurring polysaccharides from microbes via fermentation processes: Such biotechnological production can be from naturally producing organisms or, thanks to advances in gene technologies, optimised strains that have been engineered. There is expertise within larger companies in fermentation technologies, which are of course capable of being adapted for the production of particular polysaccharides and to overcome issues that are associated with scale-up, particularly for engineered systems.

- Chemical synthesis: Traditionally it has been very challenging to synthesise carbohydrates chemically. However, chemistry advances including the introduction of automated processes are now enabling the synthesis of larger and more complex polysaccharides. This is mostly carried out by expert academic research groups and specialist Small & Medium Enterprises (SMEs). Developments of more wide-ranging synthetic methodologies that can be replicated by non-specialist organic chemists are needed and improved availability of the building blocks is also required.

- Enzymatic synthesis: This is being used increasingly and is often a more sustainable approach than are traditional synthetic approaches. Moreover, recent examples demonstrate that the use of functionally optimized enzymes (engineered to apply product size control in the production process) enable the synthesis of oligo- or polysaccharide fractions ready for coupling to the protein carrier within hours[x]. However, a major limitation can be the availability of carbohydrate-active enzymes with the required properties and chemical activities. There is therefore an urgent need to build an arsenal of more glycoenzymes showing an expanded repertoire of chemistries.

- Combined integrated approaches: Using combinations of some or all of the above methods together also presents great promise (*e.g.* in sequence or in parallel chemical and/or enzymatic modification of naturally occurring polysaccharides). Such integrated approaches require cross-disciplinary strategies including chemistry, biochemistry, molecular biology and computation, and machine learning.

The issue of machine learning, alluded to above, deserves more detailed description here, because it is also here that the measurement and metrology challenges implicit in WP2 are to be found. During the production on an industrial scale of the novel carbohydrate vaccines, quality control is absolutely critical, not just so that the product could have the required bio-efficacy but also for economic, health and safety reasons (e.g., it could be that only minute quantities of certain isomeric impurities could be a severe risk to health). Therefore appropriate metrological protocols using on-line or in-line methods of rapid analysis must be built in to the manufacturing plant adopted so that detection of deviations from the required specifications of product quality are captured very quickly. This requirement in turn predicates rapid, effective analytical methods linked to cognitive computational systems which control and direct robotic manufacturing of the vaccine product (the so-called "Industry 4.0" approach). Such an approach is of course not unique to polysaccharide vaccine manufacturing but the issue addressed here is that in the case of polysaccharides the required basic metrologies are not sufficiently developed; hence the need for this Work Programme (*but see also WP5*). For glycoconjugate vaccines, oligo/polymer sizes of average degree of polymerization of up to around 15 have been shown to exhibit optimal properties in vaccine formulations. Consideration also needs to be given to the production of the conjugated protein moiety, the epitope, typically 10 amino acids in length, which is crucial for providing adequate T-cell help, and importantly the methodologies for linking the protein moiety to the polysaccharide (*see WP3 below*).

WP3. METROLOGY FOR OPTIMISING GLYCOCONJUGATE AND ADJUVANT DESIGN: Conjugate Selection and Design plus Methods for Conjugation

The choice of carrier protein and conjugation method both have impact on the overall effect of the glycoconjugate vaccine on the immune response. These choices affect not just the effectiveness of the vaccine but may also affect vaccine stability and shelf life.

There is a need for new conjugation methods that are less complicated and more cost-effective than current approaches in order to help reduce overall production costs. Methods must allow control over the modification site so the resulting conjugate is produced with consistency. These studies require a highly sophisticated level of measurement capabilities linked to well-defined metrological protocols.

Currently there is a limited number of carrier proteins that are traditionally used (e.g. DT, TT, CRM197). Since they are used in existing vaccines, safety and regulatory aspects have already been largely addressed. However in recent years there have been major efforts to identify alternative carriers, often with the dual role of carrier and antigen, so as to target both carbohydrate and protein virulence factors of a given pathogen. Successes here include the Hib vaccine (PedvaxHIB® produced by Merck) which vaccinates against *Haemophilus influenzae* type

b (Hib) infections using an outer membrane vesicle as a carrier. There is potential for other new carrier systems, such as inorganics, nano-particles, and carrier classes such as virus-like particles (VLPs), or liposomes that could be explored for their effects in an effort to overcome immune interference issues associated with existing carriers.

Availability of a wider range of carriers will allow more options for glycoconjugate vaccine design and those that enhance the action of the vaccine in a dual carrier-adjuvant role are also sought, leading to both more effective vaccines, and reduced production costs. Due to the crucial role of the carrier in glycoconjugate vaccines more basic research is required in this area (*see WP4 below*). Currently, adjuvant selection is considered more of an art rather than an exact science; WP4 will aim to address this issue in depth.

WP4. MEASUREMENT OF VACCINE MECHANISMS OF ACTION AND THEIR EVOLUTION: Fundamental Glycoimmunological Studies for Improved and Robust Vaccine Design

Further understanding of the exact and varied roles carbohydrates play in immunisation mechanisms will undoubtedly allow improved vaccine design. New and improved animal models are needed that will allow the study of bacterial diseases in humans, as well as the effect of antibiotics and how AMR arises. In addition to this, improved and more detailed mechanistic models that address fundamental understanding of the immune response are also required and it is here that advanced measurement techniques that can elucidate subtle details of the mechanistic processes must be employed, not to mention metrological systems that ensure the maintenance of required quality standards. This information will in turn allow the development in parallel of *in silico* predictions of immunization and infection which will reduce the need for many animal experiments. In addition, the role of adjuvants and their immunological modes of action in combination with carbohydrate-based vaccines needs much more study, based on sound and reproducible measurements, in order to achieve fast antibody production as foreseen for the growing problem of AMR infections not just in the future, but now.

Fundamental understanding, originating in advanced measurement of the carbohydrate and glycoconjugate antigen processing and presentation mechanisms, will also shed light on the role of the carriers and adjuvants in the overall vaccine mechanism of action and in turn allow the selection and development of different and perhaps more effective carriers, particles and adjuvants (*see WP3 above*). This may even allow the design of carrier molecules that also have intrinsic adjuvant activity.

This improved understanding also needs to include the impact of the epitope, and whilst there are various bioinformatics tools that allow major histocompatibility complex (MHC) class I predictions of an epitope's ability to elicit antibody production there is a growing need for *in silico* MHC class II prediction tools[xi] (*and see WP6 below*). For active evolution of such *in silico* tools the models will need monitoring and metrological checking on an ongoing basis in order to ensure that refinements move in a useful direction.

Similarly, ongoing monitoring and understanding of the antigenic-drift phenomenon due to environmental and pharmaceutical pressures is also necessary to ensure the efficiency of vaccines. This can be widely explored using clinical isolates and monitored through the development of appropriate databases (*see WP6 below*).

Improved fundamental understanding of the role carbohydrates play and the differences between active and passive immunisation will allow a number of challenges in carbohydrate-based vaccine development to be overcome *e.g.*:

i. Selection of 'optimal' antigens and in response, the production of monoclonal antibodies (mAB);
ii. Understanding of immune protection mechanisms;
iii. Allowing the development of strategies to tackle infections due to multiple pathogens ("super-infections");
iv. Provision of know-how into the *in situ* drug delivery process.

This fundamental knowledge of the immune system and the impact of the various components of a vaccine formulation on it, is critical for the future design of efficacious and safe vaccines. None of it can develop safely and effectively without the existence of adequate measurement and metrology capabilities.

WP5. REGULATION, ISO-STANDARDS, INTELLECTUAL PROPERTY MANAGEMENT AND MONITORING: Scaled-up Production of Carbohydrate-Based Vaccines

WP2 above refers primarily to measurement and modelling needs for accurate and reliable manufacture of polysaccharide synthesis on an industrial scale in a future Industry 4.0 manufacturing facility. WP5 here emphasises some complementary aspects to this, which are also key requirements for effective future advancement of the field. WP2 described the need for robust analytical tools that allow for in-process analysis coupled with cognitive computing and robotic manufacturing during the production of carbohydrate vaccines; however, there is similarly an urgent need that these capabilities should be developed and adapted for use by non-specialists in the manufacturing production setting). WP5 is concerned with these latter needs.

Useful in-process analytics need to provide data on various aspects which include identity, quantification, purity, impurity profile, safety/sterility, plus physicochemical and immunochemical assays (*e.g.*, through the use of mAb reagents) for quality control. These need also to address and meet regulatory requirements aimed at ensuring safety and efficacy of the vaccine product.

Regulatory guidance does exist for carbohydrate-based vaccines (USP <1234>)[xii]. The WHO also produces guidelines on vaccines alongside other agencies including the European Medicines Agency and the Food and Drugs Administration (FDA).

The challenge here is to help researchers, whether academic and industrial, but especially SMEs, to navigate effectively the regulatory landscape and to be comprehensively aware of, and meet, regulatory guidelines aimed at ensuring effective drug development. For example, FDA drug approval data showed that 2015 was a particularly productive year for the pharmaceutical industry, which the FDA attributes to better interactions with companies early on in the drug development process[xiii].

To enable more candidates to come through the development pipeline, informed support and advice to small companies should be provided on an ongoing basis as one of the objectives of this Work Package, which could take the form of analytical service provision and/or method validation as well as reference standard availability.

Concerning intellectual property and security, there are huge opportunities for larger companies where the production method is itself proprietary and therefore has potential for patent protection (rather than the vaccine itself). A recent example of success here is GlycoVaxyn, a company spun out from the Swiss Federal Institute of Technology (ETH Zurich). Its technology is focused on the production of glycoconjugate vaccines in bacterial cells, a biotechnology approach allowing the development of innovative vaccines. GSK has acquired the company and this proprietary technology has the potential to allow GSK to develop a simplified manufacturing process for the production of glycoconjugate vaccines.

WP6. INTERFACE BETWEEN INFORMATICS AND METROLOGY: Bioinformatics for Data Integration, Knowledge Sharing and Improved Vaccine Design

To manage glycoscience data and make the information accessible to the wider scientific community, a number of publicly available databases and modelling tools have been already been developed. However there is a need to integrate the information into more centralised and dedicated platforms (as for DNA and protein research and technology development) and to develop this into a long term, permanent, stable, up-to-date and open platform to support discovery projects. This is the key objective of WP6.

The ongoing MIRAGE project[xiv] "Minimum Information Required for A Glycomics Experiment" is currently working to establish guidelines for data presentation from glycomics research in a variety of outputs including for on-line database entries ensuring data quality and consistency. On-line databases also need dedicated resources for their curation.

Several reliable sources cover information on cell surface polysaccharides, such as the details of their structure in the Bacterial Carbohydrate Structure Database (BCSDB), of their binding properties in the Protein Data Bank (PDB) and of their biosynthesis/degradation properties in the Carbohydrate-Active enZymes Database (CAZy)[xv].

For effective glycoconjugate vaccine design these data need to be integrated with relevant genomic, proteomic and clinical data. This will provide an integrated knowledge-base for researchers working to develop carbohydrate-based vaccines. Additional source data for integration, for instance produced within a vaccine project consortium (but depending on confidentiality rules), also needs to be considered. At key development stages, user needs and feedback should be collected to ensure usability.

The resulting Knowledge Base would underpin and support a range of user activities including data interrogation and mining. Accurate metrological tool development for comparative purposes and ultimately for predicting structural and functional properties of surface molecules will also be key to user discovery projects. The Knowledge Base could also support other studies (e.g. antigen epidemiology studies). Currently these tools are very much in the academic realm and need to be further developed with industry in mind. Tools will need to capture standardised data to meet regulatory requirements.

WP7. METROLOGY AND THE DEVELOPMENT OF BUSINESS MODELS: Business Model for Development of Carbohydrate-Based Vaccines

As for all drug development, vaccine research requires significant investment. The recent commercial successes of the Merck HPV LP vaccines and Pfizer's Prevnar have shown that vaccines present increasingly attractive targets in the 'Biologics' market both for human and animal health. In order for companies to increase investment in this area they need a 'push' from technology developments, as well as a 'pull' from public health needs, perhaps through commitments from governments and health organisations to buy such vaccines when they become available[xvi]. Efforts are needed to provide the metrology-based advice and epidemiological evidence that vaccines are indeed a cost-effective solution.

For smaller companies, regulatory hurdles can be a bottleneck. Advice on understanding and implementing regulatory criteria needs to be more clearly provided, perhaps through business specific guidance documents. As highlighted above, the earlier on in the development process that manufacturers engage in dialogue with regulators (which should include advice on effective clinical trial design and metrology) the higher the chance of regulatory approval success. In addition, given the urgency presented by the growing AMR crisis, fast track priority review as regulatory incentives would also perhaps encourage companies to carry out more vaccines research.

This Work Package would therefore seek to ensure that adequate metrological capabilities are developed that keep pace with regulatory demands. For this, industry and governments need to work together to develop suitable business models for vaccine development. This could include public private partnership initiatives such as GAVI[xvii] which involve governments, business and charities working together in a coordinated effort.

Impact on Health, the Environment & Security

CarboMet has identified the production of carbohydrate-based vaccines as playing a crucial future role in the strategy to overcome the increasing antimicrobial resistance threat.

We contend that the key to delivering high impact using carbohydrates is simple: **it is the ability to respond quickly and effectively to disease-based threats**. The ability to produce vaccines rapidly and in scaled-up quantities in response to infectious outbreaks will make mass vaccination campaigns more feasible with accompanying positive impacts on societal health and the economy. In addition, fast and effective mass vaccination capabilities in response to the threat will reduce the danger and occurrence of epidemics, with a concomitant reduction in the need for treatments, which has implications for healthcare costs.

This is not just an issue for the development of human vaccines. It is broadly recognised that glycometrics also holds great promise for the development of carbohydrate-based vaccines against epizootic diseases. This is particularly interesting since a number of pathogens that cause animal diseases have zoonotic potential, and thus may also affect the health of human beings. (*See Appendix for list*). Thus, transfer of the above-described glycotechnologies to the veterinary field (including zoonotic pathogens) is highly relevant and should therefore also be given high priority.

An example is *Streptococcus suis*, a major swine pathogen and an emerging zoonotic agent of human meningitis and streptococcal toxic shock-like syndrome[xviii]. Although research on developing an efficient vaccine to protect post-weaning pigs from *S. suis* infection has been ongoing, this has not yet led to a commercially available vaccine. However, glycoconjugate vaccine approaches have revealed highly promising results for several *S. suis* serotypes[xix]. These proof-of-concept results demonstrate the potential of glycoconjugate vaccines in veterinary medicine applications to prevent invasive bacterial infections and consequently highlight the need to explore this technique further.

It is generally the case that veterinary vaccines can be developed and licensed much more quickly and at a lower cost than human vaccines. Keeping the costs of animal vaccines low will encourage their use and help to reduce the use of antibiotics. In this context, we are aware of the 'One Health' approach[xx] which identifies the need for an integrated effort between multiple disciplines working locally, nationally and globally in order to achieve to optimal health for humans, animals and the environment to reduce the use of antimicrobials and its implications for veterinary vaccination.

It is therefore clear that carbohydrate-based vaccines will be a future important set of health tools to protect human health, animal health, food safety, and food security on a broad front. However, speedy and effective research on such accessible, economic vaccines depends on the availability of quick, accurate and reliable measurement capabilities, which do not adequately exist at present, rendering for example previous glycoconjugate approaches generally too expensive for veterinary applications, especially for livestock farming.

Note also that the development of new technologies for carbohydrate-based vaccines will open new directions in preventing infectious diseases in animals, which is also crucial for reducing instances of zoonotic diseases, and especially for AMR pathogens. Recent success include the synthesis of complex glycan-epitopes in one-pot reactions in a cost effective approach[xxi].

The generic glycotechnologies developed in this programme would also be applicable to the development of vaccines for other diseases such as AIDS, neglected infectious diseases, and particularly cancer, which is of joint interest to the topic of this paper in cases of microbial infection-associated carcinogenesis. The potential here is enormous. For example, the market for cancer vaccines has been estimated at more than $4 billion in 2014 and is expected to reach $4.3 by 2019[xxii].

In conclusion, **CarboMet** draws attention to the huge potential of novel carbohydrate-based vaccines in fighting AMR as well as protecting both humans and animals from disease and epidemics. To exploit this huge potential adequately, new and old measurement techniques need to be brought together to solve current problems and then to drive ground-breaking research into such vaccines. Investment in novel carbohydrate measurement and metrology is an essential prerequisite for producing innovative carbohydrate-based vaccines that will fully benefit society, as specified in the Work Programmes proposed above.

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Appendix:

1. WHO Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics

(Available from http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/).

Priority 1 Critical:

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant (*Enterobacteriaceae* include: *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter spp*., *Serratia spp*., *Proteus spp*., and *Providencia spp*, *Morganella spp*);

Priority 2 High:

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter*, fluoroquinolone-resistant
- *Salmonella spp*., fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant

Priority 3 Medium:

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella spp.,* fluoroquinolone-resistant.

Notes

1. Non-bacterial infections such as HIV and malaria are not included.
2. Mycobacteria (responsible for tuberculosis) were not included as they have already been established as a global priority.

2. Veterinary targets against epizootic and/or zoonotic diseases

- *Streptococcus suis*
- *Brucella spp.*
- *Coxiella burnetii*
- *Mycoplasma bovis, Mycoplasma mycoides, Mycoplasma gallisepticum…*
- *Campylobacter jejuni**
- *Salmonella spp.**
- *Enterobacteriaceae**

**Also on WHO Global Priority list*

Glossary of Terms

Adjuvant - a substance that is mixed with an immunogen in order to elicit an enhanced immune response.

Antigen - any substance or entity (e.g., a virus or micro-organism) which is foreign to the host's body and evokes a defensive immune response.

Antibody - molecular entity that is produced within the blood of a human or animal in order to destroy specific foreign cells (*see also 'antigens'*).

Bioinformatics – the use of mathematics and computer science to collect, classify, store, retrieve and analyse biochemical and biological data.

Carbohydrates - also known as **sugars**, mono- oligo- and poly-**saccharides** and **glycans.**

Epitope - specific molecular region on the surface of an antigen which can elicit an immune response from the host organism.

Epizootic - epidemic outbreak of a disease in an animal population, with the implication that it will extend to humans e.g."bird flu"

Glycation - covalent bonding of sugars to other molecules, especially proteins, by chemical reactions not catalysed by enzymes.

Glycoconjugates - carbohydrates linked covalently to other biomolecules for example glycoproteins or glycolipids.

Glycosylation reactions are catalysed by **glycosyltransferase** enzymes that add glycosyl radicals to other molecules (usually proteins) in a site-specific manner.

Glycomics – study of the structure and function of carbohydrates in biological systems.

ISO-standards - "requirements, specifications, guidelines and characteristics that should be used to ensure that molecules, products, processes and services are fit for purpose" [https://www.iso.org/standards.html (ISO = International Organization for Standardization)].

Major histocompatibility complex ('MHC') - cluster of genes involved in the immunological recognition of "self" (i.e., the cells of an organism) and "non-self" (i.e. exogenous cells of invading organisms) encoding cell surface proteins.

Metrology - science of weights and measures, the reliable determination of conformity with product specifications and/or technical requirements as well as standards developments [see http://www.businessdictionary.com/definition/metrology.html]. ISO standards are built on sound metrological procedures. Thus metrology is not therefore synonymous *per se* with descriptions of measurement instrumentation and techniques.

Monoclonal antibody ('mAB') - antibody prepared using a laboratory-grown cell clone. It is more uniform than a natural antibody and is therefore able to bind very specifically to a particular epitope on any chosen antigen I

Operon - cluster of functionally-related genes that are controlled by a shared molecular operator.

Pathogen - any disease-producing agent, applies especially to viruses, bacteria and other micro-organisms such as fungi.

Serotype - group of organisms, micro-organisms or cells that are distinguished by their shared specific antigenic properties, as determined by serological testing.

T-cells a type of lymphocyte (white blood cell) that circulate in the body which respond to infective and/or malignant cells in the body.

Zoonotic is an infectious disease in animals that can be transmitted to humans, e.g. Ebola, rabies.