ANTIBODIES AGAINST *KLEBSIELLA PNEUMONIAE* AND IMPLEMENTATIONS THEREOF

FIELD OF INVENTION

[001] The present disclosure broadly relates to the field of immunology and vaccines and particularly relates to a process for obtaining antibodies or antigen binding proteins against *Klebsiella pneumoniae* (*K. pneumoniae*).

BACKGROUND OF INVENTION

[002] *K. pneumoniae* has been assigned as a microbe of particular concern by WHO due to its emergence as a crucial driving force in significant morbidity and mortality due to drug resistance. MDR and XDR strains of this bacteria has resulted in significant increase in hospital stays and eventual deaths (WHO. Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. Geneva: World Health Organization. 2017). Even by conservative estimates, unless novel antimicrobials are discovered, by 2050 antibiotic resistance will claim 10 million lives annually across the globe. Of this, the majority of victims will stem from Asia and Africa (O'Neill, J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance. May 2016). Wanton use of antibiotics, especially in India, where antibiotic stewardship is still in its infancy, has made the country a driving force in antibiotic resistance. Drug resistant *K. pneumoniae* ranks third in annual deaths caused by ESKAPE pathogens in the country (Koya, S.F., Ganesh, S., Selvaraj, S., Wirtz, V.J., Galea, S. and Rockers, P.C. Consumption of systemic antibiotics in India in 2019. The Lancet Regional Health-Southeast Asia 2022;4:100025). Thus, from a national and global perspective, the development of novel antimicrobials is call of the hour.

[003] Antibodies that form one of the mainstays of humoral immunity against infectious diseases are interesting candidates for the development of novel antimicrobials. One of the major reasons behind this is the specificity of antibodies

against its target antigen. Availability of recombinant and/or traditional monoclonal antibodies make them even more attractive as a therapeutic alternative against bacterial infections (Ziegler EJ, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli* N Engl J Med. 1982;307(20):1225–1230; Baumgartner JD, et al. Prevention of gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. Lancet. 1985;2(8446):59–63; Greenman RL, et al. A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram-negative sepsis. The XOMA Sepsis Study Group. JAMA. 1991;266(8):1097–1102; Ziegler EJ, et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. N Engl J Med. 1991;324(7):429–436). However, the acute specificity of monoclonal antibodies makes them an untenable option for *K. pneumoniae* as the bacteria has immense variability (12 variants of LPS and 77 variants of CPS). Thus, aneffective monoclonal therapy against clinical strains will require raising of huge numbers of monoclonal antibodies (Berry, J.D. and Gaudet, R.G. Antibodies in infectious diseases: polyclonals monoclonals and niche biotechnology. 2011. New Biotechnology. Volume 28, Number 5).

[004] Under the aforesaid circumstances, polyclonal antibodies with high efficacy against a broad spectrum of *K. pneumoniae* strains, have become highly attractive.

SUMMARY OF THE INVENTION

[005] In a first aspect of the present disclosure, there is provided a process for obtaining an antigen binding protein against *Klebsiella pneumoniae* (*K. pneumoniae*), said process comprising: (a) immunizing an equine with an antigen formulation comprising a pool of *K. pneumoniae* strains and an adjuvant; (b) collecting blood from the equine after 100–300 days, and isolating plasma from the blood; (c) diluting the plasma with saline to obtain a diluted plasma; (d) precipitating the diluted plasma of step (c) with at least one saturated fatty acid to obtain a plasma-

acid mixture; and (e) processing the plasma-acid mixture to obtain the antigen binding protein against *K. pneumoniae*.

[006] In a second aspect of the present disclosure, there is provided an antigen binding protein against *K. pneumoniae* obtained by the process as described herein, wherein the antigen binding protein is a polyclonal polyvalent antibody.

[007] In a third aspect of the present disclosure, there is provided a composition comprising the antigen binding protein or the polyvalent polyclonal antibody as described herein.

[008] In a fourth aspect of the present disclosure, there is provided a method for treatment of a disease caused by *K. pneumoniae* in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of the antigen binding protein or the composition as described herein.

[009] In a fifth aspect of the present disclosure, there is provided a method for the diagnosis of a disease caused by *K. pneumoniae* in a subject, comprising contacting the antigen binding protein as described herein with a sample obtained from the subject.

[0010] In a sixth aspect of the present disclosure, there is provided a method of detecting *K. pneumoniae* in a sample obtained from a subject comprising contacting the antigen binding protein as described herein with the sample.

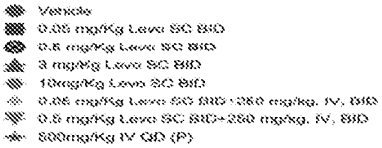
[0011] These and other features, aspects, and advantages of the present subject matter will be better understood with reference to the following description and appended claims. This summary is provided to introduce a selection of concepts in a simplified form. This summary is not intended to identify key features or essential features of the disclosed subject matter, nor is it intended to be used to limit the scope of the disclosed subject matter.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS

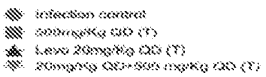
[0012] The following drawings form a part of the present specification and are included to further illustrate aspects of the present disclosure. The disclosure may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

[0013] Figure 1 depicts *in-vitro* ELISA results demonstrating antigen-antibody interaction, in accordance with an embodiment of the present disclosure.

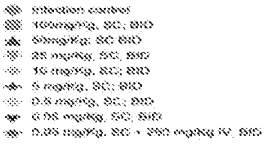
[0014] Figure 2 depicts the efficacy of treatment of *Klebsiella* infection caused by strain ATCC 43816 (Hypervirulent sensitive strain) using a combination of *Klebsiella* antibody and antibiotic Levofloxacin, in accordance with an embodiment of the present disclosure.



[0015] Figure 3 depicts the efficacy of treatment of *Klebsiella* infection caused by strain KB98 (classical MDR clinical strain) using a combination of *Klebsiella* antibody and antibiotic Levofloxacin, in accordance with an embodiment of the present disclosure.



[0016] Figure 4 depicts the efficacy of treatment of *Klebsiella* infection caused by strain ATCC 43816 (Hypervirulent sensitive strain) using a combination of *Klebsiella* antibody and antibiotic meropenem, in accordance with an embodiment of the present disclosure.



[0017] Figure 5 depicts the efficacy of combination therapy of *Klebsiella* infection in immunocompetent mice model using a combination of *Klebsiella* antibody and antibiotic meropenem, in accordance with an embodiment of the present disclosure.

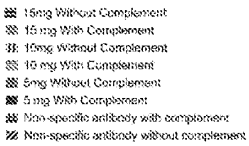
5(a)	5(b)	5(c)
Infection Control 200 mg/Kg Meropenem SC; BID 250mg/Kg Antibody, IV; BID 1mg/Kg Meropenem SC, + 150mg/Kg Antibody IV; BID	Infection Control 100 mg/Kg Meropenem SC; BID 10mg/Kg Meropenem, SC; BID 250mg/Kg Antibody, IV; BID 10mg/Kg Meropenem, SC+250mg/Kg Antibody, IV; BID	Infection control 800 mg/Kg SC Meropenem; QD 1mg/Kg SC Meropenem; QD 500mg/Kg Antibody IV; QD 1mg/Kg SC Meropenem + 500mg/Kg IV Antibody; QD

[0018] Figure 6 depicts the efficacy of combination therapy of *Klebsiella* infection in immunocompromised or neutropenic mice model using a combination of

Klebsiella antibody and antibiotic Meropenem, in accordance with an embodiment of the present disclosure.

6(a)	6(b)
Infection Control 300 mg/Kg Meropenem SC; BID 250 mg/Kg Antibody IV; TID 250 mg/Kg Antibody IV; QID 1mg/Kg SC Meropenem BID; + 250 mg/Kg Antibody, IV; TID	Infection Control 600 mg/Kg SC Meropenem; BID 600 mg/Kg SC Meropenem; QID 250 mg/Kg Antibody, IV; QID 600 mg/Kg Antibody, IV; QID 50mg/Kg SC Meropenem; BID + 250 mg/Kg Antibody, IV; QID 200 mg/Kg SC, Meropenem; + 250 mg/Kg Antibody, IV; BID
6(c)	**6(d)**
Infection Control 500 mg/Kg Meropenem, SC; BID 250 mg/Kg Antibody, IV; QID 50 mg/Kg SC, Meropenem QD; + 250 mg/Kg Antibody, IV, TID 100 mg/Kg Meropenem, SC; + 500 mg/Kg Antibody, IV QD	Infection Control 600 mg/Kg Meropenem, SC; BID 600 mg/Kg Meropenem, SC; QID 250 mg/Kg Antibody, IV; QID 600 mg/Kg Antibody, IV; QID 100 mg/Kg SC, Meropenem + 250 mg/Kg Antibody, IV; BID 80 mg/Kg SC, Meropenem BID; + 250 mg/Kg Antibody, IV; QID

[0019] Figure 7 provides *in vitro* data pertaining to end point reduction by growth inhibition assay and serum bactericidal assay, in accordance with an embodiment of the present disclosure.



DETAILED DESCRIPTION OF THE INVENTION

[0020] Those skilled in the art will be aware that the present disclosure is subject to variations and modifications other than those specifically described. It is to be understood that the present disclosure includes all such variations and modifications. The disclosure also includes all such steps, features, compositions, and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any or more of such steps or features.

Definitions

[0021] For convenience, before further description of the present disclosure, certain terms employed in the specification, and examples are delineated here. These definitions should be read in the light of the remainder of the disclosure and understood as by a person of skill in the art. The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

[0022] The articles "a", "an" and "the" are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

[0023] The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included. It is not intended to be construed as "consists of only".

[0024] Throughout this specification, unless the context requires otherwise the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated element or step or group of element or steps but not the exclusion of any other element or step or group of element or steps.

[0025] The term "including" is used to mean "including but not limited to". "Including" and "including but not limited to" are used interchangeably.

[0026] The term "equine" as used herein refers to horses or other members of the horse family. The term "equine" may be used interchangeably with the terms "horses" or "ponies".

[0027] Although the clinical trials on immunotherapy are absent, significant academic research is being done in the space of monoclonal antibodies against various epitopes of the *K. pneumoniae* bacteria. These monoclonal antibodies have shown some protection against the bacterial infections in murine models. Despite such initial success, these monoclonal antibodies suffer from the crucial disadvantage of being extremely specific towards the epitope and thus have the potential to fail against a bacterial pathogen that is as diverse as *K. pneumoniae.* There is no antibody-based therapy available for *Klebsiella* infected patients to date.

[0028] This problem can be circumvented by raising polyclonal antibodies with very wide specificity against *K. pneumoniae strains* on the whole and not specific epitopes on the bacteria. Traditional human polyclonal antibodies against infectious agents are usually raised from the serum of patients afflicted by them. The acute morbidity and mortality of *K. pneumoniae* makes serum collection from patients difficult. Additionally, since the pathogen cannot be ethically administered to healthy individuals, serum collection from volunteers is untenable.

[0029] Polyclonal antibodies derived from horses offer an attractive approach due to the possibility of rapid development timeline, scalability and availability of

infrastructure and manufacturing process. Such polyclonal antibody would exhibit high efficacy against a broad spectrum of *K. pneumoniae* strains.

[0030] The present disclosure provides a method for preparing polyclonal antibodies against *K. pneumoniae.* For immunizing the horses an antigen formulation was used and for this purpose a pool of *K. pneumoniae* strains were employed. All strains were typed according to methods well known in the art.

[0031] The polyclonal antibodies that can be raised in equines are obtained by the process of the present disclosure. The polyclonal antibodies obtained by the process of the present disclosure recognize multiple epitopes, whereas monoclonal antibodies only recognize one epitope. The polyclonal antibodies obtained by the method of the present disclosure have also proved to be highly cost-efficient as compared to monoclonal antibodies. Another disadvantage of using monoclonal antibodies is their susceptibility to variants if there are changes in the epitopes of the antigen.

[0032] According to the process of the present disclosure, the polyclonal antibodies or equine anti-serum are obtained within a very short period of time period. The equines can subsequently produce very high titers against the target antigen by administration of booster doses. The dosage for every batch can be standardized resulting in batch-to-batch consistency of potency.

[0033] The present disclosure also provides a method of manufacturing purified and concentrated therapeutic polyclonal antibody or antigen binding protein from equine plasma. In the present disclosure, nanofiltration, was used to minimise the risk of any other zoonotic transmissions. The filtration method is used for obtaining the purified and concentrated therapeutic polyclonal antibody from hyperimmunized equine plasma. The results demonstrate consistency in the quality and safety of such equine products. With the process of the present disclosure, the production of equine polyclonal antibodies can be scaled up very rapidly.

[0034] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the

disclosure, the preferred methods, and materials are now described. All publications mentioned herein are incorporated herein by reference.

[0035] The present disclosure is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally equivalent products, compositions, and methods are clearly within the scope of the disclosure, as described herein.

[0036] Embodiments herein provide a process for obtaining an antigen binding protein against *Klebsiella pneumoniae.*

[0037] In an embodiment of the present disclosure, there is provided a process for obtaining an antigen binding protein against *Klebsiella pneumoniae* (*K. pneumoniae*), said process comprising: (a) immunizing an equine with an antigen formulation comprising a pool of *K. pneumoniae* strains and an adjuvant; (b) collecting blood from the equine after 100–300 days, and isolating plasma from the blood; (c) diluting the plasma with saline to obtain a diluted plasma; (d) precipitating the diluted plasma of step (c) with at least one saturated fatty acid to obtain a plasma-acid mixture; and (e) processing the plasma-acid mixture to obtain the antigen binding protein against *K. pneumoniae*.

[0038] The process for obtaining an antigen binding protein as described herein includes a step of, immunizing an equine with an antigen formulation comprising a pool of *K. pneumoniae* strains and an adjuvant. The term "antigen binding protein" as used herein refers to a protein or any fragment thereof which us capable of binding to a site (epitope) on a target molecule, such as an antigen. The term may be interchangeably used with terms "antibody" or "immunoglobulin" or "*Klebsiella* antibody". In a preferred embodiment of the present disclosure, the "antigen binding protein" is an antibody, more preferably, a polyclonal antibody or a polyclonal polyvalent antibody. More preferably, the "antigen binding protein" is a polyvalent polyclonal IgG antibody, most preferably, the "antigen binding protein" is a *Klebsiella* IgG immunoglobulin" having a molecular weight in the range of 150-160 kDa.

[0039] The term "immunizing" as used herein refers to a procedure of administering a target protein, preferably an antigen or group of antigens to a subject

such that the subject produces an antibody against the antigen. The administration for immunization may be done using methods known in the art. A preferred method as per the present disclosure is directly injecting the antigen to a pre-prepared site of the animal, i.e., an equine. In an embodiment herein, immunizing the equine is with a dose in the range of at least $2x10^{10}$ to $100x10^{10}$ cells/ml of the antigen formulation. An equine immunized with the antigen formulation may be referred to as an "immunized equine".

[0040] In an embodiment of the present disclosure, there is provided a process for obtaining an antigen binding protein against *Klebsiella pneumoniae* (*K. pneumoniae*) as described herein, wherein immunizing the equine is with primary dose and booster dose of the antigen formulation. In another embodiment, the primary dose is in the range of $2x10^{10}$ to $100x10^{10}$ cells/ml and given in between 0-35 days. In yet another embodiment, the booster dose is in the range of $4x10^{10}$ to $64x10^{10}$ cells/ml and given in between 49-162 days.

[0041] The term "antigen formulation" as used herein is the preparation used for immunizing the equine for the production of the antigen binding protein. In an embodiment herein, the antigen formulation comprises a pool of *K. pneumoniae* strains and an adjuvant, wherein the pool of *K. pneumoniae* strains comprises subtypes of O and K antigens, preferably the subtypes are O1, O2, O3, O5, O8, K1, and K2.

[0042] In a further embodiment of the present disclosure the antigen formulation is prepared by a process comprising: (a) obtaining different strains of *K. pneumoniae;* (b) performing a serotypic analysis of the strains based on O and K subtypes; (c) studying the antibiotic resistance profile of selected strains from step (b) followed by genotypic analysis for specific resistance markers; (d) growing the selected strains of *K. pneumoniae* and pooling; and (e) preparing bacterial lysates of selected pooled strains of step (d) to obtain an antigen formulation comprising a pool of *K. pneumoniae* strains. In an exemplary embodiment, the different strains of *K. pneumoniae* comprises clinical isolates, hospital strains, and clinical strains from repositories selected from American type culture collection (ATCC), National Collection of Type Cultures (NCTC) or combination thereof. The different strains

of *K. pneumoniae* may further comprise strains derived based on genomic sequencing.

[0043] The term "serotypic analysis" as used herein refers to a process of identifying and classifying microorganisms based on their serotype (or serovar), and the same is determined by the specific antigens present on their surface. Serotypic analysis is used to distinguish between different strains or variants of a microorganism within the same species. Serotypic analysis may be done using methods well known in the art such as agglutination tests, immunoassay, molecular serotyping etc. The term "genotypic analysis" as used herein refers to a process of examining an organism's genetic makeup (genotype). Genotypic analysis may be done using methods well known in the art such as, polymerase chain reaction (PCR), DNA sequencing, restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP) analysis, microarray analysis, etc.

[0044] The embodiments of the present disclosure further provide an antigen formulation comprising a pool of *K. pneumoniae* strains as described herein or obtained from the process as described herein.

[0045] The term "adjuvant" as used herein refers to a substance that enhances the body's immune response to an antigen or target protein. In the context of vaccines, adjuvants are added to boost the effectiveness of the vaccine by stimulating a stronger and longer-lasting immune response. There are many adjuvants known in the art. Common adjuvants are aluminum salts, Freund's Adjuvants, bacterial components, synthetic polynucleotides, saponin-based adjuvants, etc. Preferred embodiment as per the present disclosure include adjuvant including but not limited to Freund's complete adjuvant (FCA), and Freund's incomplete adjuvant (FIA).

[0046] The process for obtaining an antigen binding protein as described herein includes a step of, collecting blood (comprising the antigen binding protein or the antibodies against the antigen formulation of the *pneumoniae*) from the equine after 100 – 300 days, and isolating plasma from the blood. The term "collecting" as used herein refers to the process of taking out or withdrawing blood from the equine which has been immunized with the antigen formulation, i.e., post immunization. Collecting may be done after 100-300 days of immunization. Preferably, the blood

may be collected after 120 to 250 days of immunization. More, preferably, the blood may be collected after 150 to 200 days of immunization. The collection of blood post immunization may be done at intervals, and each collection may be referred to as "bleed" and may be numbered as "first bleed", "second bleed" or "bleed 1", "bleed 2", and so on. For instance, the collection done for the first time post immunization may be referred to as first bleed. The preferable period for first bleed ranges from 150 to 180 days post immunization. The blood collected in each of the bleed may then be pooled together for final processing or may be processed individually. Preferably, the plasma isolated from the blood is pooled before further processing. Before, the actual "bleed", "test bleeds" may be done at certain intervals for detection and confirmation purposes. Collection or blood or bleed, may be done using methods well known in the art. In an exemplary embodiment, the equines are bled by venepuncture of the jugular vein.

[0047] The process for obtaining an antigen binding protein as described herein includes the steps of, diluting the plasma with saline to obtain a diluted plasma; and precipitating the diluted plasma with at least one saturated fatty acid to obtain a plasma-acid mixture. Diluting the plasma with saline may be done by adding saline to plasma in a ratio range of 1:2 to 1:4, preferably 1:3 of plasma: saline. The "precipitating" of the diluted plasma may be done using at least one saturated fatty acid. Accordingly, embodiments herein include precipitating the diluted plasma with at least one saturated fatty acid to obtain a plasma-acid mixture, wherein the saturated fatty acid is octanoic (or caprylic acid). In another embodiment, the octanoic acid is added to the diluted plasma in a concentration in a range of 3.5% to 5% v/v, preferably 4% v/v. The term "plasma-acid mixture" as used herein refers to a combination of plasma (as obtained from the immunized equines) with saturated fatty acid. In a preferred embodiment, the "plasma-acid mixture" is a combination of plasma (as obtained from the immunized equines) and octanoic (or caprylic acid). The process for obtaining an antigen binding protein as described herein includes a step of, processing the plasma-acid mixture to obtain the antigen binding protein against *K. pneumoniae.* Processing the plasma-acid mixture comprises the steps of centrifuging and diafiltration the plasma-acid mixture of step (d) of the process as

described herein, followed by purifying by chromatography to obtain a filtrate; and subjecting the filtrate to nanofiltration followed by sterile filtration to obtain the antigen binding protein against *K. pneumoniae.* According to the embodiments herein, "centrifuging" the plasma-acid mixture may be done at 700 to 1000 rpm, preferably 750 to 900 rpm, more preferably 750 to 850 rpm for a time period of 3 to 4 hours. In further embodiments the plasma-acid mixture may be subjected to diafiltration post centrifuging. In an embodiment diafiltration or diafiltering may be performed using tangential flow filtration. For tangential flow filtration, the plasma-acid mixture is passed through a cassette preferably of size 50KDa, followed by 10-15 diavolume of buffer. In one or more embodiments, the tangential flow filtration is performed using 50Kda membrane, preferably polyethylene sulfone (PES) membrane. In one of the embodiments the tangential flow filtration is performed till absorbance of the permeate or filtrate is <0.1 at 280nm.

[0048] After the centrifuging and the diafiltrating, plasma acid mixture is purified by chromatography to obtain a filtrate. The chromatography may be selected from the group consisting of anion exchange chromatography, cation exchange chromatography, hydrophobic interaction chromatography, affinity chromatography, and combinations thereof. In a preferred embodiment, the chromatography is anion exchange chromatography. The filtrate obtained post chromatography may be subjected to nanofiltration followed by sterile filtration to obtain the antigen binding protein against *K. pneumoniae.* In one or more embodiments the filtrate obtained after nanofiltration may be subjected to an optional step of diafiltering using tangential flow filtration. In another embodiment, the tangential flow filtration is performed using 50Kda membrane, preferably polyethylene sulfone membrane. In instances where a diafiltration is performed after centrifugation and another diafiltration is performed post nanofiltration, the diafiltration after centrifugation may be referred to as first diafiltration and the diafiltration after the nanofiltration may be referred to as second diafiltration.

[0049] The term "nanofiltration" as used herein refers to a membrane filtration process that uses membranes with nanometer-sized pores to separate particles based on size and charge. Nanofiltration may be done using commercially available

membranes such as hollow fibre type, tubular type, capillary type, flat sheet type, or spirally wound type. Nanofiltration allows for selective separation of a component of interest and is a robust process employed for virus removal. In the present disclosure, nanofiltration of the filtrate may be done for separating the antigen binding protein of a specific size. A further embodiment of the present disclosure includes a step of sterile filtration of the filtrate comprising the antigen binding protein after nanofiltration to obtain the final antigen binding protein against *K. pneumoniae*. Sterile filtration may be referred to a process used to remove microorganisms from a fluid stream (such as filtrate) without adversely affecting the product (the filtrate comprising the antigen binding protein). This method is particularly useful for sterilizing heat-sensitive components.

[0050] Embodiments herein provide an antigen binding protein against *K. pneumoniae* obtained by the processas described herein, wherein the antigen binding protein is a polyclonal polyvalent antibody. Polyclonal antibodies are a mixture of antibodies produced by different B cell clones in the body. These antibodies recognize and bind to multiple epitopes (specific parts of an antigen) on the same antigen. A polyvalent antibody is an antibody that can bind to multiple different antigens or multiple epitopes on the same antigen. This broad reactivity makes polyvalent antibodies particularly useful in various diagnostic and therapeutic applications. A polyclonal polyvalent antibody is highly sensitive, cost effective, can recognize multiple targets and exhibits enhanced efficiency.

[0051] An embodiment of the present disclosure provides a composition comprising the antigen binding protein as described herein and at least one excipient. An excipient as used herein may be an inactive substance used in a pharmaceutical composition to serve as the vehicle or medium for the active ingredient (such as the antigen binding protein). The excipient preferably does not interfere with the therapeutic action of the active ingredient. The role of the excipient may include but is not limited to stabilization of the active ingredient or enhance its shelf life, assisting in delivery of the active ingredient, providing the desired consistency to the composition, and/or providing the desired appearance to the

composition. In a preferred embodiment, the excipient is selected from the group consisting of sodium chloride, and glycine.

[0052] The antigen binding protein against *K. pneumoniae* as described herein, may be used in treatment of diseases caused by *K. pneumoniae* in a subject in need thereof. Accordingly, the embodiments herein provide a method for treatment of a disease caused by *K. pneumoniae* in a subject in need thereof, comprising administering to said subject a therapeutically effective amount the antigen binding protein as described herein or the composition as described herein. The treatment as described herein may be prophylactic or therapeutic. The term "therapeutically effective amount" as used herein refers to the quantity of a drug or active ingredient (antigen binding protein) that is sufficient to achieve the desired therapeutic effect in treating a disease or condition. The "therapeutically effective amount" may vary depending on several factors, the condition being treated, the patient's physiology, such as age, weight etc., as well as the route of administration. The "administration" may be done using methods or routes well known in the art such as, intravenous, intramuscular, subcutaneous, or intradermal.

[0053] The antigen binding protein against *K. pneumoniae* as described herein, may be used in diagnosis of diseases caused by *K. pneumoniae.* Accordingly, the embodiments herein provide a method for the diagnosis of a disease caused by *K. pneumoniae* in a subject, comprising contacting the antigen binding protein as described herein with a sample obtained from the subject.

[0054] In an embodiment of the present disclosure, there is provided a method of detecting *K. pneumoniae* in a sample, comprising contacting the antigen binding protein as described herein with a sample obtained from the subject.

[0055] The term "sample" as used herein refers to a sample collected from a subject, preferably human. In another embodiment, the sample may be selected from body fluids, tissue homogenates or secretions. The "sample" is suspected of comprising the antigen which may be detected or diagnosed by the antigen binding protein as described herein.

[0056] The term "diseases" as referred to herein may be selected from a group consisting of (including but not limited to) pneumonia, urinary tract infections

(UTIs), bloodstream infections (bacteremia or septicemia), meningitis, liver abscesses, wound or surgical site infections, and skin infections (cellulitis). The subject may be a mammal, preferably a human being.

[0057] The term "diagnosis" or "detection" as used herein may be done using methods well known in the art. Examples of such methods include but are not limited to, enzyme-linked immunosorbent assay (ELISA), western blotting, immunofluorescence, radioimmunoassay (RIA), agglutination tests, lateral flow assays (rapid tests) etc. Preferable methods are ELISA, western blotting, and lateral flow test.

[0058] In an embodiment of the present disclosure, there is provided a method for the treatment of a disease caused by *K. pneumoniae* in a subject in need thereof, as described herein, wherein the treatment further comprises administering at least one antibiotic. The antibiotic may be administered before, after or along with the administration of the antigen binding protein or a composition comprising the antigen binding protein. In another embodiment, the antibiotic may be selected from levofloxacin, meropenem, carbapenems, taxobactom, amoxicillin, polymixins, cephalosporins, piperacillin, or combinations thereof.

[0059] In an embodiment of the present disclosure, there is provided a combination or therapeutic combination comprising the antigen binding protein as described herein and at least one antibiotic as described herein. Also provided in the embodiments is a combination therapy using the combination of the antigen binding protein as described herein and at least one antibiotic as described herein.

[0060] Although the subject matter has been described with reference to specific embodiments, this description is not meant to be construed in a limiting sense. Various modifications of the disclosed embodiments, as well as alternate embodiments of the subject matter, will become apparent to persons skilled in the art upon reference to the description of the subject matter. It is therefore contemplated that such modifications can be made without departing from the spirit or scope of the present subject matter as defined.

EXAMPLES

[0061] The disclosure will now be illustrated with working examples, which is intended to illustrate the working of disclosure and not intended to take restrictively to imply any limitations on the scope of the present disclosure. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein. It is to be understood that this disclosure is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary.

Example 1

Selection, transportation and housing of Equines

[0062] Locally available non-descript ponies (equines) were selected. Although as mentioned in the WHO Guidelines, there is no documented case of transmission of any zoonotic infectious agents, including viral diseases through antivenoms or any other animal-derived immunoglobulins, a comprehensive Risk Control Process to be in line with International Guidelines to ensure safety of the product being administered to the patients was performed. The animals being inducted into the manufacture of antibodies were tested for various diseases mentioned in the WHO guidelines. As mentioned by the WHO the Control of infectious risks and their transmission in biological products is a result of a combination of multiple steps which include: minimizing the potential initial virus content by implementing a quality system for the production of the starting material during the process of selection of animals, monitoring of animals, controlling the manufacturing process to inactivate and/or remove residual viruses during manufacture of the biological product and introduction of dedicated viral reduction steps.

[0063] The physical eligibility criteria for the selected equines were determined based on a physical examination wherein the selected healthy equines were male, in the age bracket of 5 to 8 years, weighed at least 150kg and were at least 115cm tall. The selected equines were screened using a diagnostic blood test to check for

complete blood count, liver and kidney function tests and test for diagnosis of glander, equine infectious anaemia, strangles, trypanosomiasis, and babesiosis. The selected equines were transported to the farm.

[0064] The selected equines were transported to the farm in animal transport vehicles by maintaining proper restraining conditions and grass bedding. Adequate feed and water were arranged so as to make the transportation process comfortable for the equines.

[0065] Post transportation, the equines were checked and isolated in a designated quarantine area for a minimum of 21 days. During the quarantine period, the equines were kept under observation by monitoring and recording the health parameters and given prophylactic vaccination for trypanosomiasis, tetanus and rabies. During quarantine period, the equines were tested for various diseases and dewormed by proper anthelmintic, and their stool samples were checked for worm load.

Example 2

Preparation of the antigen formulation *K. pneumoniae*

[0066] The antigen formulation used for immunization of equines to raise-polyclonal antibodies against *K. pneumoniae* was prepared in-house. For this purpose, a pool of *K. pneumoniae* strains were used. All the strains were typed according to prevalent methods. To obtain an exhaustive pool of clinical isolates, multitudes of samples from all over the country were obtained. Besides hospital strains, clinical strains from repositories like American type culture collection (ATCC) and National Collection of Type Cultures (NCTC), were also obtained and were used as control markers for typing hospital-based strains. Serotypic analysis based on O and K types was performed and overall subtypes of O & K antigens, such as O1, O2, O3, O5, O8 and K1, K2 were finally selected (The Diversity of Lipopolysaccharide (O) and Capsular Polysaccharide (K) Antigens of Invasive *Klebsiella pneumoniae* in a Multi-Country Collection, Front. Microbiol., 12 June 2020, Sec. Infectious Agents and Disease, Volume 11 - 2020).

[0067] These strains were studied for their antibiotic resistance profile, followed by genotypic analysis. Further, a few strains with other serotypes (derived based on

genomic sequencing) were also included in the pool. Bacterial lysate was extracted and treated with proteolytic enzymes, like lysozyme and trypsin, and different buffers to obtain extracts of *Klebsiella* as antigen formulation.

Example 3

Immunization of Equines using antigen formulation

[0068] The horses (as prepared in Example 1) were immunized with the antigen formulation (as prepared in Example 2).

[0069] The region around the neck was selected as the immunization site and was washed with sufficient amount of water, shaved and disinfected. Disposable syringes and needles were used to avoid contamination.

(a) Primary Immunization:

[0070] The immunization of the horses was done as per the schedule disclosed in Table 1 below. As disclosed in Table 1, during the primary immunization, the animals were immunized with the antigen formulation prepared in Example 2 along with an adjuvant, 1ml/animal (as noted in Table 1 below). After immunization, the equines were kept under observation for any untoward reactions for a period of 72 hours. If any of the subjects displayed symptoms such as high temperature, sweating, salivation and respiratory distress, they were immediately treated.

Table 1:

Immunization	Immunization Schedule (Days)	Dosage	Adjuvant	Test Bleed	Test Bleed Schedule
1st Primary test dose immunization	0	$2*10^{10}$/ml)	FCA	NA	NA
2nd Primary test dose immunization	21	$2*10^{10}$/ml)	FIA	NA	NA
3rd Primary test dose immunization	35	$2*10^{10}$/ml)	FIA	1st test bleed	40 days after 1st Primary immunization
1st Booster test dose immunization	49	$4*10^{10}$/ml)	FIA	2nd test bleed	Post 7 days of immunization

2nd Booster test dose immunization	63	$4*10^{10}$/ml)	FIA	3rd test bleed	Post 7 days of immunization
3rd Booster test dose immunization	78	$8*10^{10}$/ml)	FIA	4th test bleed	Post 7 days of immunization
4th Booster test dose immunization	92	$8*10^{10}$/ml)	FIA	5th test bleed	Post 7 days of immunization
5th Booster test dose immunization	106	$16*10^{10}$/ml)	FIA	6th test bleed	Post 7 days of immunization
6th Booster test dose immunization	120	$16*10^{10}$/ml)	FIA	7th test bleed	Post 7 days of immunization
7th Booster test dose immunization	134	$32*10^{10}$/ml)	FIA	8th test bleed	Post 7 days of immunization
8th Booster test dose immunization	148	$32*10^{10}$/ml)	FIA	9th test bleed	Post 7 days of immunization
9th Booster test dose immunization	162	$64*10^{10}$/ml)	FIA	10th test bleed	Post 7 days of immunization
Plasma Bleeding 1.5% - [Bleed 1]	176	NA	NA	NA	NA

- FCA: Freund's complete adjuvant; FIA: Freund's incomplete adjuvant

(b) Blood Collection:

[0071] Prior to blood collection, health parameters of the immunized equines, such as temperature, respiration and heart rate were monitored and if the results were in the normal range the bleeding of equine was commenced. The equine was bled by venepuncture of the jugular vein using sterilized bleeding sets. The area near the site was thoroughly cleaned shaved and disinfected before bleeding.

[0072] The blood was collected in sterilized and graduated blood collection bottles filled with anticoagulant citrate-dextrose solution. The bottle was labelled with the identifiers such as designated ID number of the selected equine, date, and signature of the veterinarian. During bleeding a constant flow of blood was ensured, and the bottles were shaken gently to ensure proper mixing of the collected blood with anticoagulant citrate-dextrose solution. The total time for blood collection was approximately 10 to 15 minutes per animal and varied depending upon the volume

of blood to be collected. The quantity of blood collected was 1.5 % of the total body weight (for example, for an equine having body weight 200kg – blood collected was 2.5 Litres). The equine was monitored during the blood collection process and the process was discontinued in case the animal showed any signs of discomfort.

[0073] Post blood collection as per the schedule in Table 1, the needle was withdrawn, and continuous pressure was applied immediately to the puncture site till the blood flow stopped. Heparin was applied to prevent the inflammation of the jugular vein. The equines were kept under observation for at least 24 hours post blood collection. The sterilized and graduated blood collection bottles were cleaned properly using 70% isopropyl alcohol and then transferred to cold room at 2 to 8°C for at least 6 hours and not more than 24 hours to facilitate settling of blood cells and separation from plasma.

[0074] The stored blood bottles were transferred for plasma separation through dynamic pass box. The plasma separation from whole blood was performed using sterilized separation sets, under a laminar air flow unit with grade C background. A vacuum pump was used for suction of the separated plasma and the plasma was collected aseptically in sterilized tanks and labelled with the plasma lot number, group number of animals bled and the date of plasma separation. The collected plasma (anti-equine serum plasma) was transported at 2 to 8°C to Ambernath Factory.

[0075] Post plasma separation, the blood cells were reconstituted with normal saline injection to compensate the volume of plasma removed. The bottles were then transferred to the water bath with shaker until the temperature of the blood was 37°C. After attaining the temperature, the bottles were transferred through pass box to the infusion area for re-infusion of blood cells in equines.

(c) Re-infusion of blood cells:

[0076] The health parameters of the equines such as temperature, respiration and heart rate were monitored and if they were in the normal range re-infusion as commenced. The blood cells were re-infused into the animal through venepuncture using sterilized transfer sets after ensuring that the ID number of the animal matched the ID number of the blood collection bottle. During the re-infusion process, the

equine was continuously monitored, and the process was discontinued if any equine displayed signs of discomfort. Post re-infusion, the needle was withdrawn, and continuous pressure was applied to the puncture site till the blood flow stopped. Heparin sodium was applied at the jugular vein to prevent inflammation. Post re-infusion the animals were kept under observation for at least 48 hours. The details and anomalies if any during the process of immunization, blood collection and re-infusion were recorded.

[0077] Quality checks were performed by on the recorded data using the soundness examination record, quarantine record, diagnostic test reports, livestock register with animal details, prophylactic vaccination details, weighing record, grooming record, hoof care record, immunization, bleeding and re-infusion (plasmapheresis) record for individual animal, post bleeding and re-infusion (plasmapheresis) health monitoring record, master plasma record, sick animal treatment record, periodic blood testing reports, cleaning of stables record and approval from committee for the purpose of control and supervision of experiments on animals (CPCSEA) and institutional animal ethics committee (IAEC). Periodic assessments of all farms related documents was performed to ensure smooth functioning of the farms.

Example 4

Purification of antigen binding proteins or antibodies:

[0078] Post re-infusion (as described hereinabove), the equine anti-serum plasma from different animals were pooled to reach a standardized batch size. The equine anti-serum plasma was diluted using saline (1:3 ratio of plasma: saline) to obtain a diluted plasma.

[0079] The diluted plasma was purified using a precipitation step by octanoic or caprylic acid (4% v/v). Saturated fatty acids such as octanoic acid and its salts act as preservatives in the production of human biological products such as human albumin. The precipitation step allowed the immunoglobulin (IgG) and its fragments to remain in liquid phase and precipitated most of the plasma proteins thereby enhancing the purity and recovery. The resulting plasma acid mixture (or

fractionated equine anti-serum mixture) contains immunoglobulin which were superior in terms of yield, turbidity, protein aggregate and potency.

[0080] The fractionated equine anti-serum mixture was centrifuged at 25- 26°C at 800rpm for 3 to 4 hrs and the post centrifuge the clarified product was dialyzed and concentrated using a tangential flow filtration step (diafiltration). A polyethylene sulfone (PES) 50 KDa membrane was used for the tangential flow filtration, which retained the equine antibodies against *Klebsiella*, while permitting passage for other proteins and octanoic acid to permeate. Initially, the clarified product was passed through the cassette followed by 10-15 diavolume of buffer, till absorbance of the permeate is <0.1 at 280nm. The resultant filtrate or permeate was free from other proteins and octanoic acid and the concentration step minimized the volume load on the next chromatographic polishing step.

[0081] For further purification of the filtrate, the filtrate was subjected to anion exchange chromatography followed by nanofiltration using commercially available membranes such as hollow fibre type, tubular type, capillary type, flat sheet type, spirally wound that were available from manufacturers such as Asahi Kasei, Merck, Pall Corporation, Cytiva and Sartorius Corporation. Nanofiltration is a robust process employed for virus removal. This step involved nanofiltration using an Asahi filter of the equine anti-serum filtrate obtained post anion exchange chromatography at 6 mg/ml protein concentration, the pH of the filtrate was adjusted to 6.70-6.90 before nanofiltration. The purified filtrate was finally filtered by repeating the concentration step using a fresh tangential flow filtration polyethylene sulfone membrane (50 kDa). This step was used to collect the purified bulk into a formulation buffer condition.

[0082] After collection of the purified equine anti-serum bulk comprising the antigen binding protein or the antibody, it was added to a formulation buffer comprising excipients such as sodium chloride and/or glycine. Potency of the purified equine anti-serum bulk was adjusted using a dilution buffer (NaCl-Glycine, pH 6.8) and protein of the bulk was adjusted to NLT (no less than) 100 mg/ml using equine normal anti-serum bulk comprising the *Klebsilella* antibody. The final formulated bulk (formulation) was sterile filtered using 0.22µm filter and collected

and in a pre-sterilized vessel under a laminar air flow hood. The yield of antigen binding protein was observed to be between 10 -15 % and the molecular weight was 150Kda.

Example 5

Examining the antigen-antibody interaction using the purified *Klebsilella* antibody

[0083] Specificity of purified antibodies (obtained in Example 4) raised in equines were evaluated on indirect ELISA using *Klebsiella* Extract, in 96-well polystyrene microtitre ELISA strips (Nunc-immuno module, Thermo Fisher Scientific, USA). The plates were coated overnight with 2.5 x 10^{10} cells / well in carbonate buffer at 2-8°C. The plates were washed thrice with PBS-0.05% Tween 20 (SIGMA, USA) and blocked with Blocking buffer - (1%BSA with PBS-0.05% Tween 20 (SIGMA, USA) and incubated at 37°C for 1 hour. Heat Inactivated Horse sera were added to each well of the plates and incubated 37°C for 1 hour. PBS and other sera were used as controls. The liquid was aspirated, and the plates were washed three times with PBS-0.05% Tween 20 (SIGMA, USA) and incubated with secondary antibodies conjugated with Horse radish peroxidase (HRP, Bethyl) at room temperature for 1 hour. After washing three times, 3, 3', 5, 5'-tetramethylbenzidine (TMB, BIO-RAD, France) was added in each well of the plates and incubated at room temperature for 30 mins. The chromogenic reaction was terminated by adding 2N H_2SO_4 after 30 minutes. The results were read at 450 nm with a microplate reader (FLUOstar Optima, BMG LABTECH). The results of the assay are provided in Figure 1 which shows the binding ability of the purified polyclonal polyvalent antibody to different bacterial strains. It is evident from the data of Figure 1, that *Klebsiella* IgG (obtained from immunized equines as per the present disclosure) show high binding to different bacterial strains as compared to sera obtained from non-immunized animal.

Example 6

Combination of *Klebsiella* antibody with antibiotics

[0084] The *Klebsiella* antibody of the present disclosure was administered into systemic mice model to test its efficacy when combined with well-known antibiotics. The details of the experiment and result are depicted in Table 2 accompanied by Figures 2, 3 and 4 respectively.

5

Table 2:

	Hypervirulent sensitive strain	Hypervirulent sensitive strain	Classical MDR clinical strain	Hypervirulent sensitive strain
	Antibiotic: Levofloxacin			Antibiotic: Meropenem
Strain	ATCC 43816	ATCC 43816	KB98	ATCC 43816
Model	Systemic	Systemic	Systemic	Systemic
Antibiotic	0.5mg/Kg; SC, BID (1hr, 13hr)	0.5mg/Kg; SC, BID (1hr, 13hr)	20mg/Kg: SC, QD (1 hr)	0.05mg/Kg; SC, BID (1hr, 13hr)
Antibody	250mg/Kg; IV, BID (0.5 hr, 12.5 hr)	250mg/Kg; IV, BID - 2 Days (0.5 hr, 12.5 hr)	500 mg/Kg; IV, QD (0.5 hr)	250mg/Kg; IV, BID (0.5 hr, 12.5 hr)
%Survival	60%	80%	80%	80%
Mean Survival	177.6 ± 57.8 hrs	209.3 ± 64.1 hrs	220.8 ± 40.5 hrs	192.0± 65.7 hrs
Antibody - Prophylactic	250 mg/Kg: IV: QD (24 hr pre-infection)	NA	NA	NA
% Survival	80%	NA	NA	NA

[0085] It can be observed from the data depicted in Table 2 and Figures 2, 3 and 4 that a treatment (prophylactic as well as therapeutic) comprising antibody of the

present disclosure combined with an antibiotic increased the survival of the animals. In particular, a combination of antibody and antibiotics helped reduce the dosage of the antibiotic in the course of treatment.

Example 7

Comparison between treatments using *Klebsiella* antibody (antigen binding protein) alone and combination of *Klebsiella* antibody with antibiotics

[0086] The present examples provide a comparison between treatments carried out using (1) *Klebsiella* antibody (antigen binding protein obtained by the method as described herein) alone, (2) well known antibiotics alone, and (3) a combination of the antibody with well-known antibiotics.

[0087] The study was carried out in both immunocompetent and immunocompromised mice (neutropenic) models obtained from commercial sources.

[0088] The schedule of the treatments along with the observations are provided in the Table 3 and Table 4 below, for immunocompetent and immunocompromised mouse models, respectively.

Table 3:

Immunocompetent Mice Model										
Strain	Infection	Inoculum (Dosage)	Antibiotic Alone		Antibody Alone		Combination Therapy			
			Concentration (mg/kg) (Antibiotic)	Protection (%)	Concentration (mg/Kg) (Antibody)	Protection (%)	Combination (mg/Kg) (Antibiotic+Antibody)	Protection (%)	Inference (Times Reduction in antibiotic)	Figure number
ATCC 43816	Systemic (IV)	(Less)10^8	200 (BID)	100	250 (BID)	80	1 (BID) +100 (BID)	100	200	Figure 5(a)
		(Severe)10^8	300 (BID)	100	250 (BID)	40	10 (BID) +250 (BID)	100	30	Figure 5(b)
K988	Intratracheal	(Lethal)10^8	600 (QD)	100	500 (QD)	90	3 (QD) +500 (QD)	90	600	Figure 5(c)

Table 4:

Neutropenic Mice Model										
Strain	Infection	Infection (Dosage)	Antibiotic Alone		Antibody Alone		Combination Therapy			
			Concentration (mg/Kg) (Antibiotic)	Protection (%)	Concentration (mg/Kg) (Antibody)	Protection (%)	Combination (mg/Kg) (Antibiotic+Antibody)	Protection (%)	Inference (Times Reduction in antibiotics)	Figure Number
ATCC 43816	Systemic	10^8	300 (BID)	100	250 (TID)	60	1 (BID) + 250 (TID)	100	300	Figure 6(a)
VB0644		10^8	600 (BID)	80	250 (QID)	20	300 (BID) + 250 (BID)	100	3	Figure 6(b)
4858	Intratracheal	10^8	500 (BID)	80	250 (QID)	40	100 (QD) + 500 (QD)	100	10	Figure 6(c)
VB0644		10^8	600 (BID)	70	250 (QID)	0	300 (BID) + 250 (BID)	60	10	Figure 6(d)

QD: Once

BID: Twice

TID: Thrice

QID: Quadruple

[0089] The result for the observation illustrated in Table 3 and 4 are also depicted in the graphs provided in Figure 5 (5a, 5b and 5c) and Figure 6 (6a, 6b, 6c, and 6d). It is apparent from the Tables as well as the Figures that by using the antibody obtained by the process of the present invention in combination with the antibiotic, the effective amount of the antibiotic used for dosing decrease drastically as compared to when the antibiotic is used alone.

[0090] It can therefore be concluded that the antibody, when used in combination with the antibiotic (combination therapy), enhances the antibiotic's effectiveness. This means that a lower dose of the antibiotic is needed to achieve the same therapeutic effect. The significant reduction in the required antibiotic dosage can have several benefits, including minimizing potential side effects, reducing the risk of antibiotic resistance, and lowering treatment costs.

Example 6

End point reduction by Growth Inhibition Assay and Serum Bactericidal Assay (SBA):

[0091] The serum bactericidal assay (SBA) is a laboratory test used to measure the ability of antibodies, in the presence of complement (baby rabbit complement), to kill bacteria. This assay is particularly important for evaluating the effectiveness of vaccines and the functional activity of antibodies induced by infection or vaccination.

[0092] The equine antibody of the present invention with or without complement and at different concentrations was tested for their ability to reduce the growth of different bacterial strains:

- Strain 1: ATCC 43816
- Strain 2: KB98
- Strain 3: ATCV 1705

[0093] Further, a comparative non-specific antibody was also used in this study.

[0094] The result for the above study is depicted in Figure 7. It can be observed that all the strains showed a reduction in growth in antibody concentration dependent manner. Further, the percentage reduction in growth was lower when complement was present. Therefore, the presence of a complement did not enhance the effect of antibodies.

[0095] With regards to the comparative non-specific antibody, it can be observed that these antibodies exhibited much lower reduction in bacterial growth as compared to the antibodies of the present invention. Thus, it can be concluded that the antibody of the present invention is specific.

Advantages of the present disclosure:

[0096] The present disclosure provides a process for obtaining an antigen binding protein, particularly, polyclonal polyvalent antibody against *Klebsiella pneumoniae* (*K. pneumoniae*). The disclosed process can be used to produce very high titers of antibodies with high purity against *K. pneumoniae*, in a quick and efficient manner by administration of booster doses. The antigen formulation that has been used for the immunization of the equines and subsequent production of antibodies, has been prepared strategically to include an exhaustive pool of clinical isolates. This is done to ensure that the polyvalent polyclonal antibody is able to bind to multiple strains

of *K. pneumoniae.* The purified antibodies can be combined with different class of antibiotics for the effective treatment of diseases caused by MDR/XDR *K. pneumoniae* strains.

[0097] Overall, the present disclosure provides a cost-effective and time-efficient process that provides the polyclonal antibodies on a large scale for effective management of diseases caused by *K. pneumoniae.*

[0098] A combination therapy using the antibodies developed through the described process herein along with the well-known antibiotics, significantly enhances the effectiveness of the antibiotic. This combination therapy approach allows for a drastic reduction in the antibiotic dosage required, offering multiple clinical and economic benefits, such as:

- Clinical Benefits: Patients may experience fewer side effects and better overall outcomes with the combination therapy.
- Antibiotic Resistance: Lowering the dosage of antibiotics can help in the fight against antibiotic resistance, a major global health concern.
- Cost-Effectiveness: Reducing the amount of antibiotic needed can make treatments more affordable and accessible.

I/We Claim:

1. A process for obtaining an antigen binding protein against *Klebsiella pneumoniae* (*K. pneumoniae*), said process comprising:

(a) immunizing an equine with an antigen formulation comprising a pool of *K. pneumoniae* strains and an adjuvant;

(b) collecting blood from the equine after 100 – 300 days, and isolating plasma from the blood;

(c) diluting the plasma with saline to obtain a diluted plasma;

(d) precipitating the diluted plasma of step (c) with at least one saturated fatty acid to obtain a plasma-acid mixture; and

(e) processing the plasma-acid mixture to obtain the antigen binding protein against *K. pneumoniae*.

2. The process as claimed in claim 1, wherein the adjuvant is selected from Freund's complete adjuvant (FCA), or Freund's incomplete adjuvant (FIA).

3. The process as claimed in claim 1, wherein the pool of *K. pneumoniae* strains comprises subtypes of O and K antigens.

4. The process as claimed in claim 3, wherein the subtypes are O1, O2, O3, O5, O8, K1, and K2.

5. The process as claimed in claim 1, wherein immunizing the equine is with a dose in the range of at least $2x10^{10}$ to $100x10^{10}$ cells/ml of the antigen formulation.

6. The process as claimed in claim 1, wherein the at least one fatty acid is octanoic acid.

7. The process as claimed in claim 1, wherein the processing comprises centrifuging and diafiltrating the plasma-acid mixture of step (d), followed by purifying by chromatography to obtain a filtrate; and subjecting the filtrate to nanofiltration followed by sterile filtration to obtain the antigen binding protein against *K. pneumoniae.*

8. The process as claimed in claim 7, wherein the chromatography is selected from the group consisting of anion exchange chromatography, cation

exchange chromatography, hydrophobic interaction chromatography, affinity chromatography, and combinations thereof.

9. The process as claimed in claim 8, wherein the chromatography is anion exchange chromatography.

10. The process as claimed in claim 1, wherein the antigen binding protein is a polyclonal antibody.

11. The process as claimed in claim 1, wherein the antigen binding protein is a polyvalent polyclonal IgG antibody, preferably a *Klebsiella* IgG immunoglobulin having a molecular weight in the range of 150-160 kDa.

12. The process as claimed in claim 1, wherein the antigen formulation is prepared by a process comprising:

(a) obtaining different strains of *K. pneumoniae*;

(b) performing a serotypic analysis of the strains based on O and K subtypes;

(c) studying the antibiotic resistance profile of selected strains from step (b) followed by genotypic analysis for specific resistance markers;

(d) growing the selected strains of *K. pneumoniae* and pooling; and

(e) preparing bacterial lysates of selected pooled strains of step (d) to obtain an antigen formulation comprising a pool of *K. pneumoniae* strains.

13. The process as claimed in claim 12, wherein different strains of *K. pneumoniae* comprises clinical isolates, hospital strains, and clinical strains from repositories selected from American type culture collection (ATCC), National Collection of Type Cultures (NCTC) or combination thereof.

14. An antigen binding protein against *K. pneumoniae* obtained by the process as claimed in claim 1, wherein the antigen binding protein is a polyclonal polyvalent antibody.

15. The antigen binding protein as claimed in claim 14 for use in treatment of diseases caused by *K. pneumoniae* in a subject in need thereof.

16. The antigen binding protein as claimed in claim 15 for use in diagnosis of diseases caused by *K. pneumoniae*.

17. An antigen formulation comprising a pool of *K. pneumoniae* strains obtained from the process as claimed in claim 12.
18. A composition comprising the antigen binding protein as claimed in claim 14, and at least one excipient.
19. The composition as claimed in claim 18, wherein the at least one excipient is selected from sodium chloride, or glycine.
20. A method for treatment of a disease caused by *K. pneumoniae* in a subject in need thereof, comprising administering to said subject a therapeutically effective amount the antigen binding protein as claimed in claim 14 or the composition as claimed in claim 18.
21. The method of treatment as claimed in claim 20, wherein the treatment is prophylactic or therapeutic.
22. The method of treatment as claimed in claim 20, wherein the treatment further comprises administering at least one antibiotic.
23. A combination therapy comprising a therapeutically effective amount of the antigen binding protein as claimed in claim 14 and at least one antibiotic.
24. The method of treatment as claimed in claim 22 or the combination therapy as claimed in claim 23, wherein the antibiotic is selected from levofloxacin, meropenem, carbapenems, taxobactom, amoxicillin, polymixins, cephalosporins, piperacillin, or combinations thereof.
25. A method for the diagnosis of a disease caused by *K. pneumoniae* in a subject, comprising contacting the antigen binding protein as claimed in claim 14 with a sample obtained from the subject.
26. A method of detecting *K. pneumoniae* in a sample obtained from a subject comprising contacting the antigen binding protein as claimed in claim 14 with the sample.
27. The method as claimed in claim 25 or 26, wherein the sample is selected from body fluids, tissue homogenates or secretions.