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Original article

Development of lipid nanoparticles (LNP)-RNA vaccine against *Streptococcus pneumonia* gene cluster facilitating the synthesis of the capsular polysaccharide in Egypt.

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ABSTRACT

Background: Meningitis and pneumonia caused by Streptococcus pneumonia are serious overwhelming infections nowadays in Egypt. Immunity is long lasting. Mortality rate of pneumococcal pneumonia occurs annually worldwide was estimated to be about 10% despite antimicrobial treatment. Ahigher mortality rate was found in patients with pneumococcal bacteremia, as it reached approximately 25 in the past forty years. **Objectives:** Development of lipid nanoparticles(LNP)-RNA vaccine against Streptococcus pneumonia gene cluster facilitating the synthesis of the capsular polysaccharide in Egypt. **Methodology**: In the present screening experimental study, LNP-mRNA vaccine against Streptococcus pneumonia gene cluster facilitating the synthesis of the capsular polysaccharide could be produced. The lipid nanoparticle vaccine delivery system size was 85 nm. Immunogenicity was assessed in animal testing stages; then, during clinical trials phases 1/2. **Results:** The vaccine showed 91% efficacy during animal testing while it was 88% in clinical trials stages 1/2. It showed superior biological activity and fewer side effects than other standard vaccines. The efficacy was long lasting due to production of powerful neutralizing antibodies against gene cluster proteins facilitating the synthesis of the capsular polysaccharide of 23 serotypes of Streptococcus pneumonia. **Conclusion:** The vaccine, in our study was effective as prophylaxis against that bacterial infection. It needs to be updated periodically to overcome the problem of high mutation rate.

Introduction

Pneumococcal disease is the leading cause of death in children under the age of 5 y globally. Every year, global children less than 5 y of age suffer from an estimated 14.5 million episodes of serious pneumococcal infectious disease and 826 000 pneumococcal related deaths, which accounted for 11% of all deaths in this age group. This continues to be a significant problem in developing countries where approximately 90% children less than 5 y of age live.1 Humoral (antibody-mediated) immunity is directed primarily against (I) exotoxin-mediated diseases such as tetanus and diphtheria, (II) infections in which

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virulence is related to polysaccharide capsules (e.g., pneumococci, meningococci, Haemophilus influenzae). Although humoral (antibody-mediated) immunity is an important host defense against many bacterial and viral diseases, in many other bacterial infections (especially intracellular infections such as tuberculosis) and viral infections, it is primarily the cell-mediated arm that imparts resistance and aids in recovery. Furthermore, cell-mediated immunity is important in defense against fungi, parasites.2 Streptococcus pneumonae is an important global pathogen that causes a wide range of clinical disease in children and adults. Pneumococcal pneumonia is by far the common presentation of noninvasive and invasive pneumococcal disease and affects the young, the elderly, and the Immunocompromized disproportionately. Patients with chronic pulmonary diseases are also at higher risk for pneumococcal infections.3 Substantial progress over the century has been made in the understanding of pneumococcal immunobiology and the prevention of invasive pneumococcal disease through vaccination.4 Currently, two pneumococcal vaccines are available for individuals at risk of pneumococcal disease: the 23-valent pneumococcal polysaccharide vaccine (PPV23) and the 13-valent pneumococcal protein-conjugate vaccine (PCV13).5 The goal of pneumococcal vaccination is to stimulate effective antipneumococcal antibody and mucosal immunity response and immunological memory.6 Vaccination of infants and young children with pneumococcal conjugate vaccine has led to significant decrease in nasal carriage rates and pneumococcal disease in all age groups.7 As new pneumococcal vaccine recommendations are being followed, continued efforts are needed to address the vaccine efficacy in the waning immunity of the ever-aging population, the implementation of vaccines using two different vaccines under very specific schedules and their real world clinical and cost effectiveness, and the development of next generation pneumococcal vaccines.8 Diseases: The most common diseases are pneumonia and meningitis in adults and otitis media and sinusitis in children.9 Characteristics: Gram-positive "lancet-shaped" cocci in pairs (diplococci) or short chains. Alpha-hemolytic. Catalase-negative. Sensitive to bile and optochin in contrast to viridans streptococci, which are resistant. Prominent polysaccharide capsule.10 85 serotypes based on antigenicity of polysaccharide capsule.11 One of the three classical encapsulated pyogenic bacteria (Neisseria meningitidis and Haemophilus influenzae are the other two). Habitat and Transmission: Habitat is the human upper respiratory tract.12 Transmission is via respiratory droplets. Pathogenesis: Induces inflammatory response. No known exotoxins. Polysaccharide capsule retards phagocytosis.13 Antipolysaccharide antibody opsonizes the organism and provides type-specific immunity.14 IgA protease degrades secretory IgA on respiratory mucosa, allowing colonization.15 Viral respiratory infection predisposes to pneumococcal pneumonia by damaging mucociliary elevator; splenectomy predisposes to sepsis.16 Skull fracture with spinal fluid leakage from nose predisposes to meningitis.17 Laboratory Diagnosis: Gram-stained smear and culture. Alpha-hemolytic colonies on blood agar. Growth inhibited by bile and optochin.18 Quellung reaction occurs (swelling of capsule with type-specific antiserum). Serologic tests for antibody not useful. Latex agglutination test for capsular antigen in spinal fluid can be diagnostic. Treatment: Penicillin G. Low-level and high-level resistance is caused by alterations in penicillin-binding proteins. No -lactamase is made.19 Prevention: Two vaccines are available. The one used in adults contains capsular polysaccharide of the 23 serotypes that cause bacteremia most frequently. The other, which is used primarily in children under the age of 2 years, contains capsular polysaccharide of 7 serotypes coupled to carrier protein (diphtheria toxoid). Oral penicillin is used in Immunocompromized children.20 The aim of the current study was the construction of novel mRNA vaccine against lethal infectious agent pneumococci worldwide.

METHODOLOGY

Ethical statement: In the current investigation, we postdated all pertinent national, institutional, and/or international standards for using both people and animals. All procedures used in the study, including those involving people and animals, were approved with approval number P-9-1-2020 by the local government, the Ethical Committee for Human and Animal Handling at Cairo University (ECAHCU), and the Pharmacy Faculty, University of Cairo, Egypt in accordance with the recommendations of the Weatherall report. Every attempt was made to minimize the number of people and animals used in the study as well as their suffering.

Source of animal models: One hundred male transgenic mice weighing 45-50 gm implanted with human lung cells were obtained and sanctioned for legalization from the pharmacology and toxicology department of the faculty of pharmacy, Cairo university, Egypt.

Inclusion criteria for animal models: Adult male mice weighing 45-50 gm which can be induced by meningitis and pneumonia infectious diseases after inoculation with Streptococcus pneumonae with an infectious dose of 1*107TCID50 through an intranasal route of administration such as transgenic mice humanized by lung human cells for increasing expression of bacterial proteins and evoking strong humoral and cell-mediated immunity. Incubation time ranged from 4-6 days for the appearance of symptoms to occur.

Exclusion criteria: Young mice and Pregnant female mice.

Sample collection:

The blood samples were collected from 100 infected patients with meningitis and pneumonia caused by Streptococcus pneumonae in different locations in Egypt.

Material:

All chemical and biochemical materials were purchased from Algomhoria pharmaceutical company, Cairo, Egypt, and Alnasr pharmaceutical company, Abo zabal Alkhanka, Qalyobia, Egypt.

Place and date of the study:

This study was done in the faculty of pharmacy, at Cairo University, Egypt between January 2020 and April 2023.

Type of study:

Screening experimental study.

METHODS

Construction of mRNA transcripts of cluster gene facilitating the synthesis of Streptococcus pneumonae capsule:

[DynabeadsTM mRNA purification kits with Catalog number:61006 were obtained from Invitrogen Thermo Fisher Scientific, USA]

The potential open reading frames for the coherent, conserved structural and functional proteins of gene cluster encoding the synthesis of the capsule of Pneumococci was identified by bio-informatics though NCBI website. The coherent structural and functional mRNA transcripts of cluster gene locus encoding the proteins responsible for construction of Pneumococci capsule of 23 valent serotypes were purified by ion-pair reversed-phase high performance liquid chromatography[IPR-HPLC] in a single step method. The purification of mRNA transcripts was performed with the aid of mRNA kits purchased from Invitrogen, USA according to the instructions of the manufacturer of mRNA kits;21 then, were enclosed with lipid nanoparticles bubbles.These lipid nanoparticles were formed of 50 mcg of dimethyl dioctadecyl ammonium bromide lipid(DDAB). DDAB, a Quaternary ammonium lipid that complex-ed with mRNA and induced innate immunity forming vesicles that encapsulated mRNA transcripts. The particle size of the lipid nanoparticles vaccine delivery system was approximately 85 nm. Northern blot technique was utilized to determine the sizes and quantities of different mRNA transcripts of 23 valent serotypes encoding capsule formation of Streptococcus pneumonae. Northern blot first used denaturing gel to separate mRNA transcripts according to the size. mRNA was then transferred into a nylon membrane; while, keeping the same distribution in the gel. After fixing the mRNA transcripts to the membrane, labeled probe complementary to the gene of interest was then added to hybridize immobilized RNA. The non-specifically bound probes were then washed away. The solid membrane with the probe specifically bound to mRNA transcripts of interest was then dried, exposed, and analyzed. Northren blot determines the abundance and the seizes of the transcripts of mRNA of interest.22

Upstream and downstream processes the recombinant of mRNA transcripts:

For mRNA transcripts immunizing agents production, genes[cDNA] encoding target protein of interest(cluster gene encoding proteins facilitating the synthesis of Pneumococcal capsule formation) were extracted from 23 valent Pneumococci serotypes threw national centre of biotechnology[NCBI] website; then, amplified using polymerase chain reaction[PCR]; afterwards, inserted into PET-21(+) transcription vector which afterwards, was transformed in Escherichia coli BL21(DE3) expression host[both expression vector and expression host were obtained from Novagen company,Germany]. The mRNA transcripts encoding the protein of interest were purified by high performance liquid chromatography[IPR-HPLC] in a single step. Moreover mRNA transcripts were enclosed with lipid nanoparticles vaccine delivery system with particle size 85 nm. IPTG was

added after the transformation of the transcription vector into the host expression to induce DE3 bacteriophage to secrete recombinant T7 RNA-polymerase for the initiation of transcription process. NO protein expression occur due to the lack of expression vector. mRNA transcripts were only the gene products due to only usage of transcription during this process. During mRNA vaccine production, there were neither cell lines nor bacterial fermentation heavily utilized. The purity of mRNA transcripts was also, assessed using the absorbance ratio between wavelengths of 260 and 280 nm. Esherchia coli DH5α[Stratgene] was used as the primary strain host for the construction and propagation of transcription vector PET-21(+). Minimal medium 9[M9] and broth were used for routine bacterial culture at incubated temperature 37 0C for 24 hours. The antibiotics[Kanamycin A and Ampicillin] were added to the media according to the refrences recommendations. T7 Lac promoter was utilized during recombinant process inside expression host strain Escherichia coli BL21(DE3).23

Formulation:

mRNA in lipid nanoparticles vaccine. The dosage form was intramuscular injection of the sterile suspension of mRNA transcripts of gene encoding the proteins facilitating the synthesize of Pneumococcal polysaccharide capsule. Each 1 ml dose contained 4 mcg of the mRNA transcript for each Streptococcus pneumonae cluster gene encoding proteins which aid in the synthesis of the polysaccharide capsule of 23 valent pathogenic serotypes/ ml, 50 mcg of the lipid dimethyl dioctadecyl ammonium bromide (DDAB), and 0.7 mg of aluminium hydroxide. Each dose also contained 0.723 mg of sodium dihydrogen phosphate dihydrate and 5.9 mg of sodium chloride.

Immunogenicity in animal models was detected by injecting the purified LNP-mRNA vaccine in 100 male transgenic mice weighing 45-50 gm by intraperitoneal route of administration.

In vitro evaluation of vaccine on transgenic animals(mice):

Transgenic mice were those whose genes were altered by recombinant DNA technology using tissue culturing technique. A transgenic animal is one that has integrated a gene of DNA sequence(a trans-gene)which has been transferred by human intervention into the genome of a cell.

100 transgenic mice were injected with the vaccine. They received two doses, 28 days were apart from each other. The first dose was half the second booster dose.24 The infective dose of Streptococcus pneumonae was observed to range from 107-108 CFU. The pathogenic twenty three valent serotypes of Pneumococci include[1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F].

Screening and bio-assay of the biological activity and toxicological effects of the vaccine.

Protection tests :

were used to determine the potency of vaccines.

Active:

Following immunization with the vaccine that was being tested, groups of transgenic mice were challenged with increasing numbers of

microorganisms.The lowest number of microorganisms lethal for 50% of animals (I.e., LD50) was determined and compared to LD 50 in

non-vaccinated animals in order to measure the protective power of the vaccine.25

Passive:

Graded amounts of serum from immunized individuals were transferred to normal mice,which are then challenged with the infectious agent .The highest dilution of serum effective at protecting 50% of animals (I.e.ED50%) was determined as a measure of the efficacy of the vaccine.26

ELISA for detection of neutralizing antibodies to the test vaccine:

[Invitrogen coated and instant ELISA kits with product number 37581 obtained from Thermo Fisher scientific company, USA]

10 µl of Antigen suspension / well was coated onto the wells by passive adsorption and incubation for 1 hour. Bovine serum albumin was used to block the other binding sites. The plates were washed with PBS-T three times to remove unbound molecules. Biotinylated Antibody (the enzyme conjugated antibody) IgG with 50 µl Horseradish peroxidase (HRP)/ well was added and incubated for 15 minutes. The wells were washed again with PBS-T to remove unbound molecules. 50 µl Chromophore substrate (TMB)/ well was added for 15 minutes which detected the presence of the enzyme and thus the antigen.27

Flow cytometry for detection of CD+4 and CD+8 T lymphocytes:

mRNA gene cluster vaccine-specific CD+4 and CD+8 T cells were seen and investigated using an Invitrogen Attune Cytpix flow cytometer (obtained from the USA). In this experiment, a monoclonal antibody was used to tag the patient's cells. These antibodies were directed against cells-specific proteins (e.g. CD4 protein when measuring the number of T helper cells). Fluorescent dyes like rhodamine and fluorescein were used to tag monoclonal antibodies. The laser beam travelled through individual cells, causing them to glow. Using a device called as a Fluorescence Activated Cell Sorter, the fluorescence was evaluated (FACS).28

Evaluation of test vaccine via human randomized clinical trials phases 1/2:

In the present investigation, 3 sets of human volunteers were used. Each group contained 100 participants:

Group 1 (the negative control group) received an intramuscular injection of the placebo.

Group 2 (the positive control group) received intramuscular injections of the common 23 valent Streptococcus pneumonae vaccination.

The test LNP-mRNA Streptococcus pneumonae vaccine was given intramuscularly to Group 3 (the test group). After two weeks, the three groups were exposed to varying concentrations of the pathogenic bacteria such as Streptococcus pneumonae to induce the development of protective neutralizing antibodies (this was ethical and was sanctioned to assess the efficacy of the test vaccine). The three groups received intradermal booster dosages after 21 days. Over the course of two years, the test vaccine's level of protection was assessed. While the protective cell-mediated immunity was assessed using a flow cytometry technique, the protective antibodies were discovered using an enzyme-linked immunosorbent (ELISA) assay.

Randomized human clinical trials:

Stage 1: In a phase 1 trial, a limited number of healthy human volunteers are used to carefully assess the new drug's pharmacokinetics and dose-response relationship (eg, 20–100). The phase 1 trials of highly hazardous medications and cancer chemotherapy are an exception; in these trials, the agents are given to willing patients who are suffering from the target ailment. Phase 1 studies examine the agent's immediate effects at a wide range of dosages, starting at a level that has no discernible impact and working up to one that either triggers a major physiologic response or has only a very modest harmful effect.

Stage 2: In a phase 2 trial, a medicine is assessed in a reasonable number of ill individuals (for example, 100–200) who have the target disease. A single-blind or double-blind design includes a placebo or positive control medication. Patients are continuously watched during the trial, which is conducted under strict controls and frequently in a hospital research unit. The objective is to ascertain whether the agent has the requisite efficacy (i.e., generates a sufficient therapeutic response) at levels that sick patients may tolerate. The pharmacokinetics and pharmacodynamics of the medication in this patient population are thoroughly studied. Stage 3:

A phase 3 trial often entails a large number of patients (1000–6000 or more, at numerous sites), as well as a large number of doctors, who are administering the drug in the method suggested for its eventual general use (e.g., in outpatients). Such studies often use a double-blind crossover design and incorporate placebo and positive controls. The objectives are to examine the new drug's range of beneficial effects in more detail under the circumstances of the proposed clinical use, to compare it to placebo (a negative control) and older treatments (a positive control), and to identify any toxicities that may occur infrequently enough to be missed in phase 2 studies. These investigations typically cost a lot of money and involve the collection of extremely huge amounts of data. Sadly, only a small number of phase 3 trials incorporate the current.

Stage 4: Phase 4 of the evaluation process is the post-marketing surveillance phase, where it is hoped that very rare toxicities would be identified and reported in time to avert catastrophic therapeutic catastrophes. All recorded adverse medication reactions must be reported to the FDA by the manufacturer at regular intervals. Phase 4 has historically been less strictly controlled by the FDA than the other three phases. There is currently a great deal of interest in improving phase 4 surveillance because so many medications have been shown to be unacceptably harmful only after they have been commercialized.

Statistical analysis

All cultures were conducted in triplets.Their presentation was by means and

standard deviation.One way analysis of variance (p value≤ 0.05) was used as means for performing statistical analysis and also, statistical analysis based on excel-spreadsheet-software. F test was utilized during this study.

RESULTS

Wherever the mRNA vaccine against Pneumococci was tested on animals, the vaccine showed 91% efficacy in preclinical trial phases, while it showed 88% efficacy during phases 1 and 2 of randomized human clinical trials. Nearly 1010 cfu were found to be the Pneumococci LD50%. It was discovered that the mRNA Pneumococci vaccine's ED50% was 92 mcg/ml. The mRNA vaccine formulation contained 4 mcg of mRNA transcript of cluster gene of each of the 23 valent pathogenic Pneumococci per millilitre. Phases 1 and 2 of randomized human clinical trials involved 85 infected participants in the negative control group, 21 infected participants in the positive control (standard) group, and 12 infected participants in the test group. It demonstrated more biological activity and fewer adverse reactions than other common vaccinations. Due to the high rate of mutations, the vaccine needs to be updated on a regular basis despite its long-lasting efficacy. When measured with a UV spectrophotometer, the ratio of mRNA transcript absorbance at 260 and 280 nm was almost 2, indicating the high purity of recombinant mRNA transcripts that were purified using the high performance liquid chromatography method.

Figure 5 represents that cytotoxic T. lymphocyte count after the immunization against Pneumococcal infection was increased significantly indicating the presence of moderate cell mediated immunity in human randomized clinical trials stages 1/2. Table 5 displays T. lymphocyte count after vaccination against Pneumococcal infection. Table 4 represents the counts of alive and dead mice the vaccination of transgenic mice with RNA pneumococcal vaccine. Table 2 represents protection power of mRNA pneumococci vaccine. Table 3 It represents the absorbance of different serum neutralizing antibodies to Pneumococcal RNA vaccine via ELISA. Figure 7 shows positive Gram stain test for Streptococcus pneumonae under the normal light microscope. Arrows point to that the clear area around the organism is the capsule. Figure 6 indicates that the count of the helper T. lymphocytes was raised mildly during randomized human clinical trials phases 1/2 reflecting the mild stimulation of cell mediated immunity. Figure 1 represents mRNA of gene cluster proteins facilitating the synthesis of the capsular polysaccharide of Streptococcus pneumonae via Northern blot technique. The percentage of purity reached approximately 80%. Figure 4 represents the absorbance of different serum concentrations of neutralizing antibodies to Pneumococcal RNA vaccine via ELISA. Figure 2 displays that the protection power of mRNA Streptococcus pneumonae vaccine reached 91% during animal testing stages. Figure 3 represents that protection power of mRNA Pneumococci vaccine was 88% during clinical trials stages1/2.

Bioinformatics analysis:

The cluster genes encoding CPS ABCD proteins involved in the formation of a system that modulated the termination of polysaccharide elongation were conserved and coherent among all Pneumococci capsule serotypes and have orthologues in other encapsulated Streptococci such as Streptococcus agalactiae. CPS A was involved in transcription of CPS operon; whereas, CPS C and Cps D played a central role in the polymerization and export of capsular polysaccharide. CPS B was a cognate phosphate. CPS BCD constituted a phosphoregulatory system where Cps D was an auto-kinase. CPS A was noticed to be 485-aa membrane protein with a major extracellular portion. The extracellular domain was proposed to be responsible for the hydrolysis of the pyrophosphate linkage between the CPS and the membrane lipid anchor and subsequent attachment of capsule[CPS] to the peptidoglycan. The cluster genes encoding CPS ABCD proteins were 1450-1455 base pairs. CPS ABCD proteins exhibited type 1 transmembrane proteins with extracellular and intracellular domains. They displayed a moderate homology[61-63%] with lip A and lip B gene products of Neisseria meningitidis facilitating surface localizarion and lipidation of capsular polysaccharide.

Table 1. List of instruments:.

Instrument	Model and manufacturer
Autoclaves	Tomy, Japan
Aerobic incubator	Sanyo, Japan
Digital balance	Mettler Toledo, Switzerland
Oven	Binder, Germany
Deep freezer -70 ^{0}C	Artikel
Refrigerator 5	Whirpool
PH meter electrode	Mettler-toledo, UK
Deep freezer -20 ^{0}C	whirlpool
Gyratory shaker	Corning gyratory shaker, Japan
190-1100nm Ultraviolet-visible spectrophotometer	UV1600PC,China
Light(optical) microscope	Amscope 120X-1200X, China

Table 2. It represents protection power of mRNA pneumococci vaccine

	Vaccinated		Nonvaccinated
Description	**Test**	**Standard**	**Control**
Infected%	12	21	85
Noninfected%	88	79	15

Table 3. It represents the absorbance of different serum neutralizing antibodies to Pneumococcal RNA vaccine via ELISA:

Concentration(ng/ml)	**Absorbance**
1.25	0.174
3.12	0.265
6.11	0.388
12.7	0.515
26	1.032
49	1.907
97	3.0446

Table 4. It represents the counts of alive and dead mice the vaccination of transgenic mice with RNA pneumococcal vaccine:

Description	Vaccinated	Non-vaccinated
Alive	91	27
Dead	9	73
Total	**100**	**100**

Table 5. It displays T. lymphocyte count after vaccination against Pneumococcal infection:

Description	Vaccinated	Pre-vaccinated
CD+4 COUNT[Ku/l]	1045	1000
CD+8 COUNT[Ku/l]	725	600
Total	**1770**	**1600**

Figure 1. It represents mRNA of gene cluster proteins facilitating the synthesis of the capsular polysaccharide of Streptococcus pneumonae via Northern blot technique. The percentage of purity reached approximately 80%.

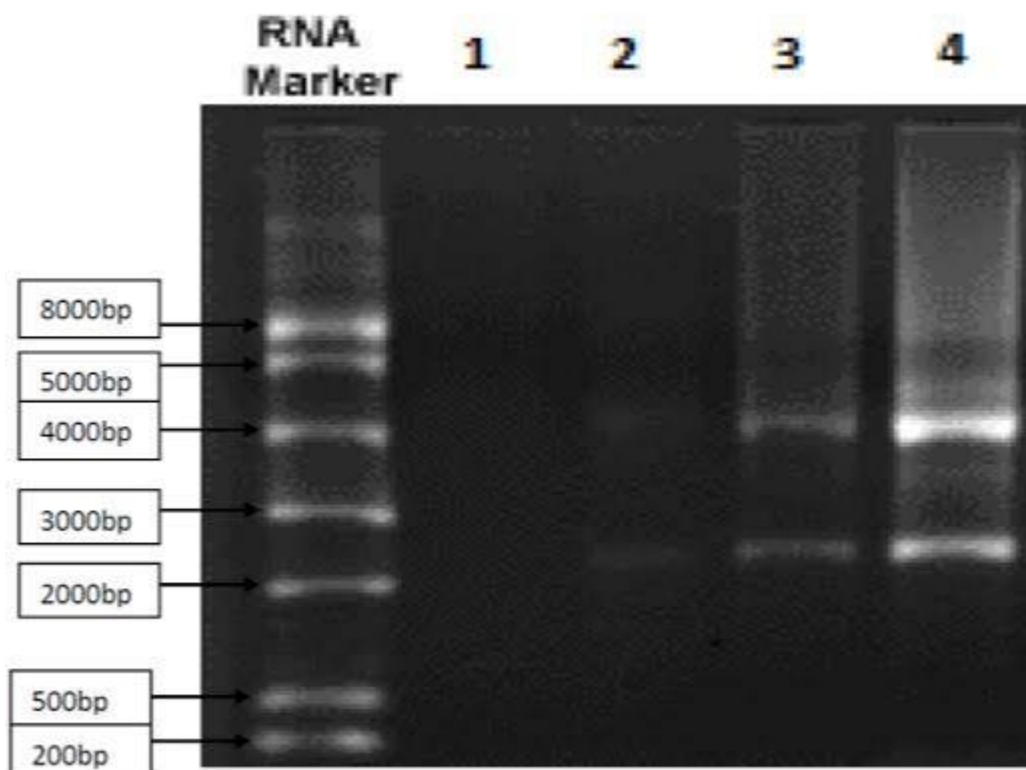


Figure 2. It indicates that throughout phases 1/2 of clinical testing, the mRNA Pneumococci vaccine's level of protection was 88%.

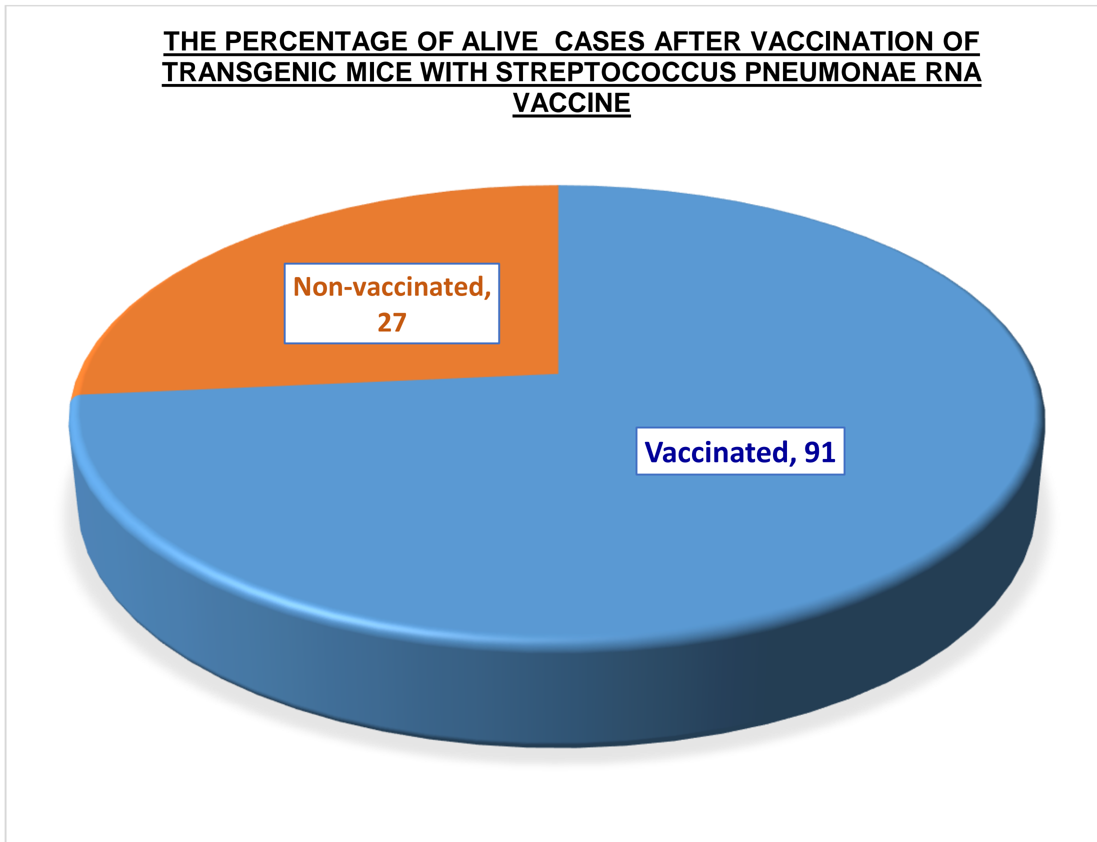


Figure 3. It indicates that throughout phases 1/2 of clinical testing, the mRNA Pneumococci vaccine's level of protection was 88%.

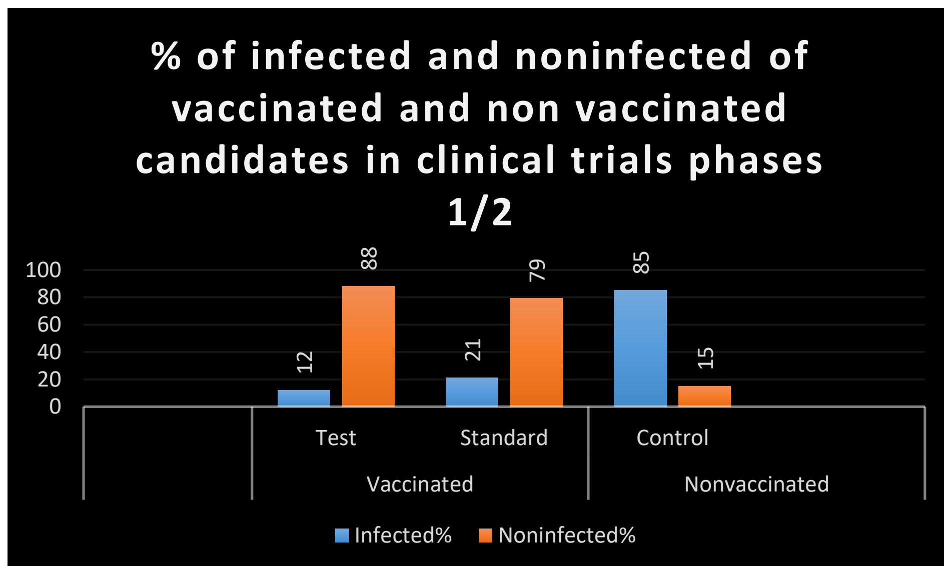


Figure 4. It shows the absorbance of various serum levels of antibodies that can neutralize the Pneumococcal mRNA vaccination using an ELISA.

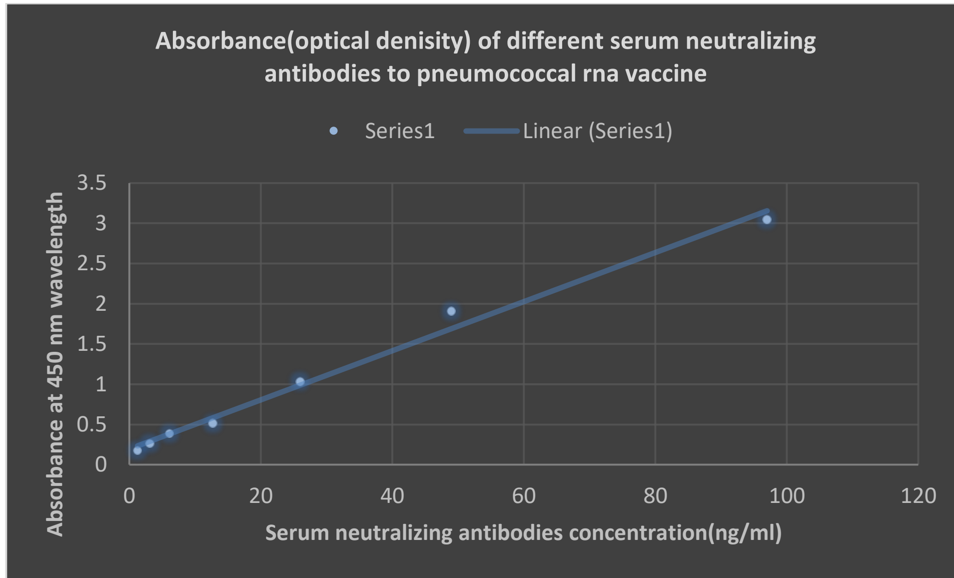


Figure 5. It indicates that the cytotoxic T lymphocyte count rose considerably following immunization against pneumococcal infection, demonstrating the presence of considerable cell-mediated immunity in human randomized clinical trials phases 1/2.

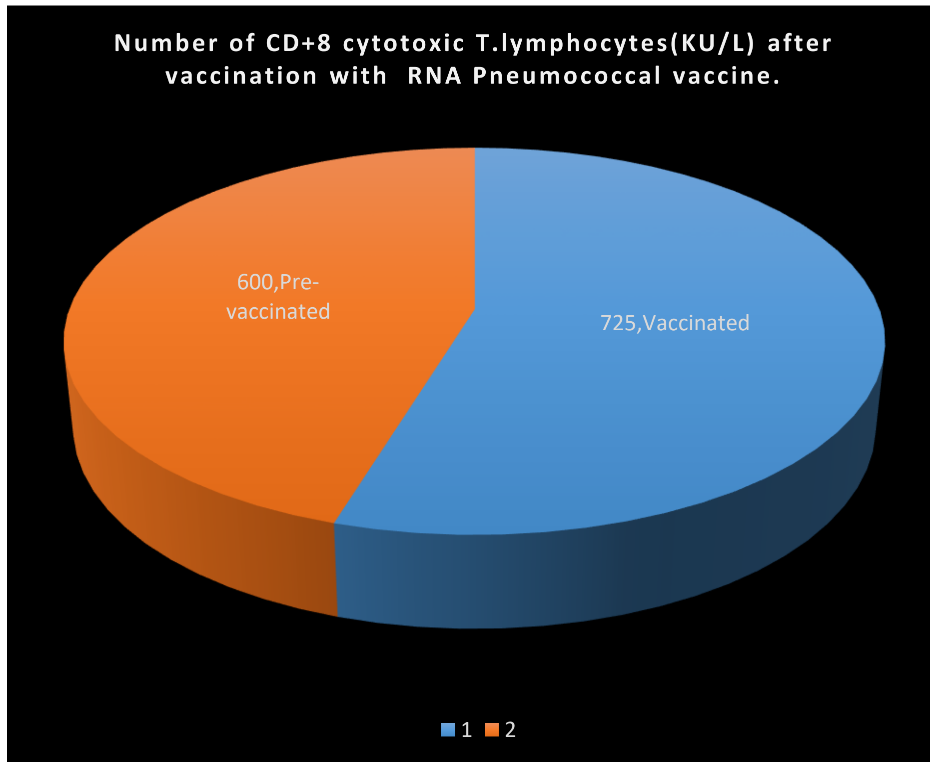


Figure 6. It shows that throughout the first two rounds of randomized human clinical trials of mRNA Pneumococci vaccine, the number of helper T cells was moderately increased, implying a minimally stimulated cell-mediated immune response.

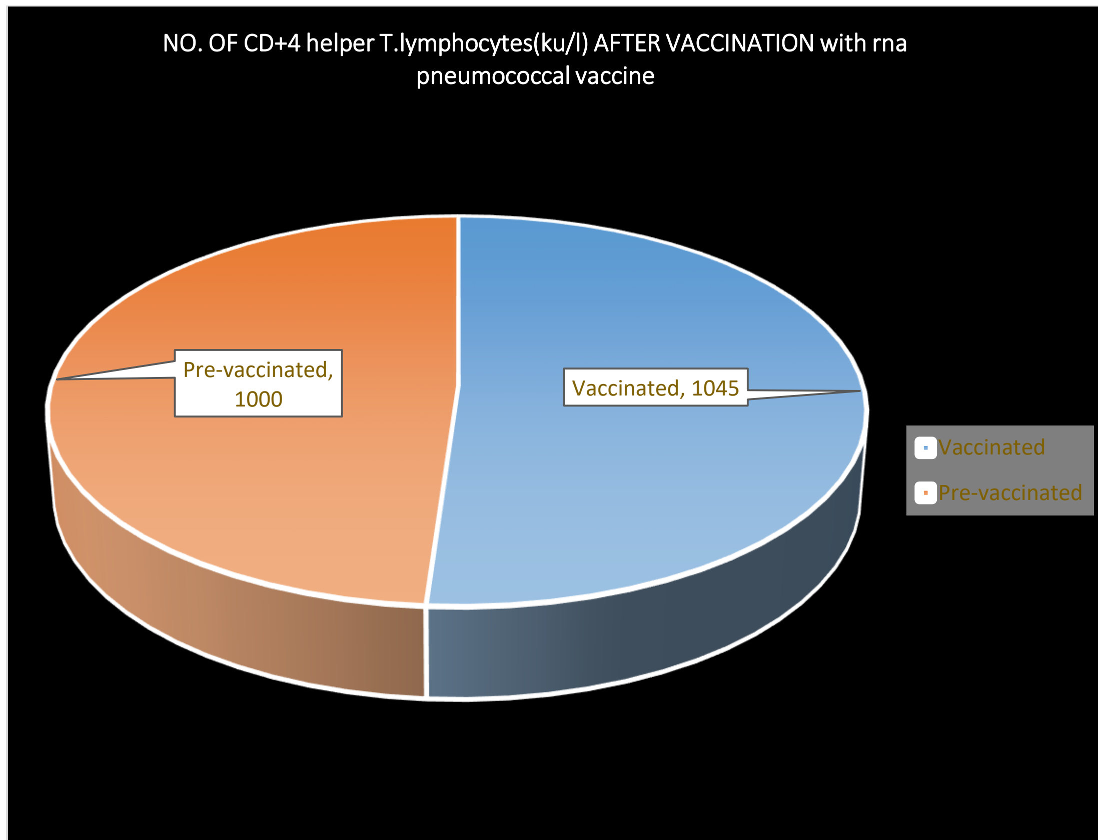
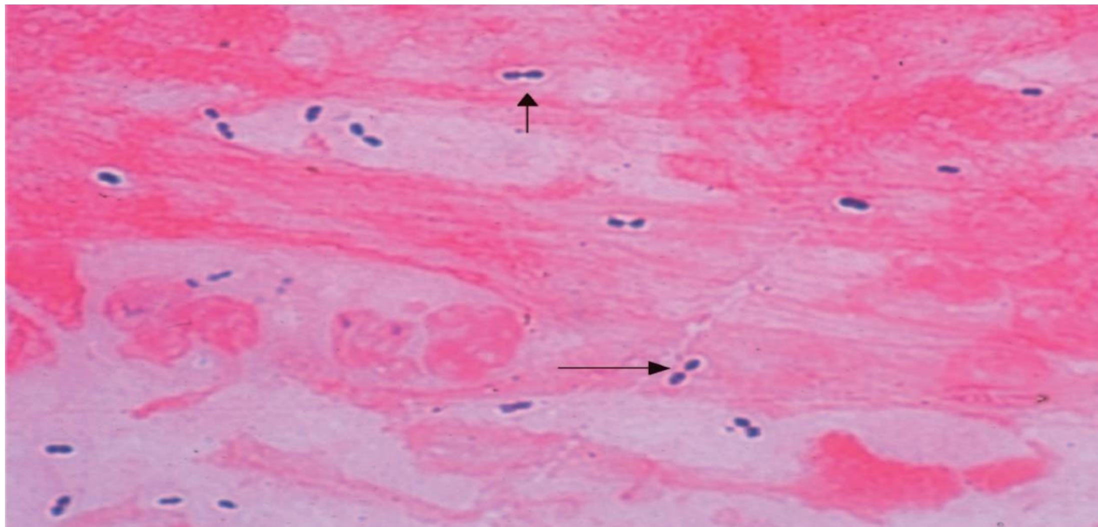


Figure 7. Under a regular light microscope, it displays positive Gramme stain results for Streptococcus pneumonae. Arrows indicate that the capsule is in the open space surrounding the creature.



Discussion

The current study aimed at the construction of lipid nanoparticles mRNA Pneumococcal vaccine to limit the lethal hazards of 23 valent serotypes of Pneumococci especially among infants younger than 2 years old. The previous vaccines initially comprised one type that was efficacious for people older than 2 years of age but with poor efficacy against infants younger than 2 years of age due to evoking no powerful neutralizing antibodies against the polysaccharide capsule which represents the main virulent agent of the infectious agent. This was stated by [Gyu- Lee Kim et al, 2017] study.29 Streptococcus pneumoniae causes pneumonia, bacteremia, meningitis, and infections of the upper respiratory tract such as otitis media, mastoiditis, and sinusitis. Pneumococci are the most common cause of community-acquired pneumonia, meningitis, sepsis in splenectomized individuals, otitis media, and sinusitis. They are a common cause of conjunctivitis, especially in children. Pneumonia often begins with a sudden chill, fever, cough, and pleuritic pain. Sputum is a red or brown "rusty" color. Bacteremia occurs in 15% to 25% of cases. Spontaneous recovery may begin in 5 to 10 days and is accompanied by development of anticapsular antibodies. Pneumococci are a prominent cause of otitis media, sinusitis, mastoiditis, conjunctivitis, purulent bronchitis, pericarditis, bacterial meningitis, and sepsis. Pneumococci are the leading cause of sepsis in patients without a functional spleen. Most pneumococci are susceptible to penicillins and erythromycin, although significant resistance to penicillins has emerged. In severe pneumococcal infections, penicillin G is the drug of choice, whereas in mild pneumococcal infections, oral penicillin V can be used. A fluoroquinolone with good antipneumococcal activity, such as levofloxacin, can also be used. In penicillin allergic patients, erythromycin or one of its long-acting derivatives (e.g., azithromycin) can be used. In the United States, about 25% of isolates exhibit low level resistance to penicillin, primarily as a result of changes in penicillin-binding proteins. An increasing percentage of isolates, ranging from 15% to 35% depending on location, show high-level resistance, which is attributed to multiple changes in penicillin-binding proteins. They do not produce β-lactamase. Vancomycin is the drug of choice for the penicillin-resistant pneumococci, especially for severely ill patients. Ceftriaxone or levofloxacin can be used for less severely ill patients. However, strains of pneumococci tolerant to vancomycin have emerged. Strains of pneumococci resistant to multiple drugs have also emerged.

Despite the efficacy of antimicrobial drug treatment, the mortality rate of pneumococcal infections is high in immunocompromised (especially splenectomized) patients and children under the age of 5 years. Such persons should be immunized with the 13-valent pneumococcal conjugate vaccine (Prevnar 13). The immunogen in this vaccine is the pneumococcal polysaccharide of the 13 most prevalent serotypes conjugated (coupled) to a carrier protein (diphtheria toxoid). The unconjugated 23-valent pneumococcal vaccine (Pneumovax 23) should be given to healthy individuals age 50 years or older. These vaccines are safe and effective and provide long lasting (at least 5 years) protection. Immunization of children reduces the incidence of pneumococcal disease in adults because children are the main source of the organism for adults and immunization reduces the carrier rate in children. A booster dose is recommended for (A) people older than 65 years who received the vaccine more than 5 years ago and who were younger than 65 years when they received the vaccine, and (B) people between the ages of 2 and 64 years who are asplenic, infected with human immunodeficiency virus (HIV), receiving cancer chemotherapy, or receiving immunosuppression drugs to prevent transplant rejection. A potential problem regarding the use of the pneumococcal vaccine is that of serotype replacement. Will the vaccine reduce the incidence of disease caused by the serotypes in the vaccine but not the overall incidence of pneumococcal disease because other serotypes that are not in the vaccine will now cause disease? In fact, an increase in invasive pneumococcal disease caused by serotype 19A, which was not in the previously used 7-valent vaccine, occurred. This led to the production of the current conjugate vaccine containing 13 serotypes, including 19A. The test mRNA vaccine resulted in 91% efficacy in preclinical trials phases; while, it was 88% during clinical trials phases 1/2. It showed superior biological activity and fewer side effects than other current standard vaccines [as shown in tables 2,4]. Its efficacy is suggested to last for approximately 5 years and the vaccine needs to be updated periodically due to the high rate of mutations of the pathogen. The ratio of the absorbance of mRNA transcripts at 260 and 280 nm using a UV spectrophotometer was nearly 2 indicating the high purity of recombinant mRNA transcripts which were purified by the high performance liquid chromatography[HPLC] method. Capsular polysaccharides [CPS] conjugated to carrier proteins are currently used as vaccines against 23 valent Pneumococci with high efficacy and immunogenicity due to the production

of powerful neutralizing antibodies which prevent the capsule of the pathogen from inactivating complement deposition and opsonophagocytosis. As well, the current test mRNA pneumococcal vaccine showed marked reduction in mortality rate as compared between vaccinated and non-vaccinated candidates during randomized human clinical trials phases 1/2. Moreover, the efficacy and immunogenicity of the test immunizing agent in the present study reached approximately 88%; while, the previous standard vaccine efficacy and immunogenicity did not exceed 85%. This was according to [Andrew C et al, 2016] study.30 The present LNP-mRNA Pneumococcal vaccine showed higher efficacy and immunogenicity than the standard current 23 valent Pneumococcal serotypes vaccine. The present vaccine did not need a carrier protein to be effective in infants older than 6 months. The immunogenicity of the test mRNA vaccine was increased obviously during preclinical animal testing; as well as, during randomized human clinical trials phases 1/2. The reason for increasing immunogenicity was due to the rise in the count of both kinds T. lymphocytes as shown in table 5. The count of CD4+ T. lymphocytes exceeded 1000 Ku/l; while, the count of CD8+ T. lymphocytes exceeded 720 Ku/l. On the other hand there was a significant rise in titters of the of the powerful neutralizing antibodies to proteins facilitating the synthesis and the transport of Pneumococcal polysaccharide capsule formed in-vivo human cells after immunization with mRNA 23 valent Pneumococcal vaccine in randomized human clinical trials stages 1/2.

The role of immunity in prevention of the infection:

This vaccine strongly stimulated the humoral immunity and moderately stimulated the cell mediated immunity.

The humoral immunity was the main body defense mechanism that prevented the infection. The main neutralizing antibodies in blood were IgM,IgG against cluster proteins involved in the formation of the capsule of Streptococcus pneumonae.Few IgA antibodies were produced because it was not taken by the natural route of infection.This vaccine weakly activated cell mediated immunity.

Advantages:

No reversion to virulence is possible.

Disadvantages:

Excretion of vaccine and transmission to non-immune contacts is not possible, thus it does not contribute to the development of the herd immunity against this bacterial infection.

Shorter duration of action than live attenuated vaccine.

It needs to be stored at -70 0C in a refrigerator to avoid spoilage and contamination.

LNP-mRNA pneumococcal vaccine is suggested for people older than six months of age. The recommended dosage of this vaccine was 92 mcg/ml dose given intramuscularly followed by a booster dose after 28 days given intradermally. In presence of 0.6mg aluminum hydroxide as an adjuvant, the immunogenicity increased from 88% to 90%. The test vaccine was contraindicated for persons having allergies against its components. The adverse effects noticed were mild pain at the site of intramuscular and intradermal injections and mild fever for a few days which were relieved by simple analgesics such as paracetamol and ketoprofen. Present vaccine contained mRNA of the cluster genes encoding the proteins responsible for the formation of the polysaccharide capsule of 23 valent pathogenic Pneumococci which was The main mode of prevention. Gene products of cluster genes are regarded responsible for lipidation and possibly surface localization of Pneumococci polysaccharide capsule. Gene products of cluster proteins are localized in the outer bacterial membranes of the pathogen. They represent type 1 transmembrane alpha helical proteins. They show a great homology with lip A and lip B gene products of Neisseria meningitidis facilitating surface localizarion and lipidation of capsular polysaccharide.

Conclusion

RNA vaccine of genes responsible for the formation of capsule of Pneumococci was effective as prophylaxis against bacterial infection with meningitis and pneumonia caused by this pathogenic bacteria .It can be taken for individuals of all ages.we suggest to explore contentiously new approaches for the development of new vaccines against the mutant types of whenever appear.

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