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Immune Characterisation of Human Local Pneumotropic Isolates of *Streptococcus pneumoniae* Serotype 1 and 6 as Local Vaccine Strains

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

S. pneumoniae is one of the principle human pneumopathogen. Such pathogenicity features in man can be delineated by severity, capsule nature and yield of pure growth onto primary plate cultures. While in laboratory animal models, it accounts for; the nature of capsule serotype, shortness of time for the death end point and extent of tissue pathology. The virulent serotypes C1 and C 6 of human pneumotropic *S. pneumoniae* were immune characterized as vaccine strains. Capsules were separated, identified and quantified. Then dispensed as 10mg deried amounts in ampoules. On reaching specific immune priming of rabbits the ampoules dissolved in 10 mls sterile saline and

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each ml admixed with 1ml lanoline and injected intramuscularly per each rabbit's thigh muscles. The immune primed rabbits left for 28 days then test bleed to check for an evident immune conversion then, at the day 35 after immunization the immune rabbits were challenged with live *S. pneumoniae* 1x 10 to six CFU/ml., in a rate of 2 mls. The carbohydrate based capsule prototype monvalent vaccines were found as; Pure, safe, antigenic, immunogenic and immune protective to the rat of 80% for C1 and 60% for C6 serotype in rabbit models. They induced systemic humoral immune precipitins responses higher than mucosal pricipitin responses. Proinflammatory cytokine responses TNF alpha, TNF beta and IL6 and anti-inflammatory cytokine IL10 were mounted as mucosal responses higher than the systemic responses. The proved immune characteristics of these capsule serotypes were suggestive for use of these strain as vaccine strains along with other serotypes in development of the local prototype multiserotype vaccines of this pathogen.

Keywords: Antigenicity; cytokine; immunogenicity; immune efficacy; proinflammatory precipitins; vaccine strain.

1. INTRODUCTION

The nature of capsule serotype in *S. pneumoniae* played as a determinal factor in pathogenesis, pathogenicity and immunogenicity [1-10]. *S. pnenumoniae* in our previous work in this area, have shown inter and intraserotype variations in pathogenicity to mice and rabbits [10]. So far the immune characterisation of the sertype 1 and 6 is concerned as a vaccine strains [10]. There were few bacterial specific carbohydrate based vaccine that are approved for mass vaccination in human clinical practice of bacterial infectious diseases. These vaccines are avialable in use for those at risk human beings by the vaccine approval authorities [1]. Such as that of multiple carbohydrate based vaccine of *S. pneumoniae* [2-10]. Though at times human pneumococcal pneumonia reported every now and then at diferent part of the developing world countries [10]. So local human pneumotropic *S. pneumoniae* isolates gained prevelence may express different pathogenic and immunogenic potentials. Such differences may be from the same serotypes present in the current internationally approved vaccine [10,11]. The present paper was aims at tempts to immune characterize human pneumotropic local serotype 1 and 6 *S.pneumoniae* isolates as local vaccine strains.

2. MATERIALS AND METHODS

Seed Strains: A collection of eight serotypes of *S. pneumoniae* that were recovered from clinical human cases of pneumonia. The study strains were elected following certain criteria both in man and laboratory animals. In man, serotype nature, severity while in animal model, serotype nature, shortness in death end point time and extent of tissue pathology. The most virulent of which were serotype 1 and 6 so they were eleted for immune characterisation as vaccine strains [10].

Laboratory Development: The 24 hr freshly revived original isolates of each serotype 1 and 6 [author collection [10] of the *S. pneumoniae* serotype 1 and 6 growth were made onto blood agar plates. Sterile heavy swab inocula were made onto 20 trypicase soy agar plates for each serotype. The inoculated plates were incubated at 5-10% CO2 tension at 37C for 24 hrs. Pure growth were harvested by 5 ml sterile saline solution per each plate. The suspensions were brought to PH 8.5 by the addition of 10% KOH. The alkalinized suspensions were heated at 90C for 1hr in water bath. These heated suspensions let stand to cool and acified with 5N acetic acid. The acidified suspensions then centrifuged at 5000 rpm for 10min.Pellets were discarded and supernatant s were mixed with two volumes of 1% sodium acetate in 95% ethanol solution then kept at 4C for 24 hrs. Supernate and pellets were formed. The formed precipitates were removed by centrifugation at 2500rpm for 10 min. Pellets were discarded and supernatants were mixed with 5% sodium actate in 0.5 N acetic acid for 24 hrs at 4C. These mixed solutions were centrifuged at 5000 for 30 min. Supernatant discarded and pellets were washed with 85%,95% absolute ethanol. Then dried at 37C. The 20 plate yeilds 200 mg powder materials. These materials were cautiousely dispensed in 10 mg per ampoule under aceptic precautions and kept till use. Each ampoule dissolved in 10 mls., sterile saline representing the immungenic doses for 10 rabbits and stands as prototype monovalent capsular carbohydrate based experimental vaccine in rabbits [12].

Purity: A purity checks were done by quadrate streak method onto trypticase soy agar plates in

each step of the laboratory developmennt of the prototype monovalent pneumococcal vaccine [13].

Safety: A 0.1 ml of the capsule experimental prototype vaccines were IM injected into rabbits through thigh muscles three replicates for each serotype. The injected animals were followed for up to five days. Then eviscerated to test for gross and histology changes [13].

Adjuvant: Lanolin solution [13].

Immunogens: One volume of lanolin was admixed with one volume of 1mg/ml. capsular polysaccharide. The mixture stands as the test immunogens [13].

Immunization Protocol: The immunogen 2 ml mixture was IM injected into the thigh mscle of rabbit and left for 15 days then test bleed [13].

Laboratory Animals: A group of Newzeland rabbits were acclimatzed for housing conditions for two weeks and kept ad libitum cnodition during specific immune priming and challenge experiments. These rabbits were categorized into the following groups;

Safety group.....6 Rabbits
Serotype 1 specific immune priming...10Rabbits
Serotype 6 specific immune priming...10 Rabbits
Sham Control .....10 rabbits

Challenge Models: The specific immune primed rabbit groups at the day 22 postpriming were challenged with live *S. pneumoniae* in strenght of 1x10 to six /m, survivivors were scored 21 days post challenge [14]

Blood Sampling: Test and control rabbits groups were subjected to blood collection by cardiac puncture. Sera were saved, dispensed at 0.5 ml alqouts in appendroph tubes and kept at -20 till test for antibody and cytoikine levels [15]

Tracheal Mucosal Globulins: Parts of the challenged and control rabbits trachea were incised and open up into sterile petri –plates. Tracheal mucasa were scrapped into the plates then 5 ml formal normal 0.5% saline were added to the scraps and mixed thorughly and tubbed into centrifuge tubes. Scraps saline tubes were centrifuge at 5000 rpm for 10 min. Supernatants were kept for processing of mucosal globulin separations in accordance with the method [16]. Pellets were discarded

Immune Investigations: Determinations of antibody response levels were done by precipitation tests as in [17,18]. Cytokine response measurements were done as the manufaturer instrutions.

Efficacy Percent Difference Statistics: Let the efficacy percent of Serotype 1 Is a and Serotype 6 is b .So the Efficacy difference percetage is:

$$[a-b]/[a+b/2] \times 100$$

3. RESULTS

Purity: All of the laborsatory development purity checks onto trypticase soy agar plates were found of negative growths.

Safety: Neither gross nor histologic changes were noted in the inoculated safety check rabbits.

Antigenicity: One from each of the vaccine lot ampoules were used for check of antigenicity test by precipitation tests.

3.1 Immunogenicity

Humoral Antibody Responses: The mean of serum and mucosal antibody titres for the challenged rabbits were 400 and 20 for serotype 1 and 213.33 and 13.33 for serotype 6. Systemic responses were higher than mucosal responses, Table 1.

Cytokine Responses: Mucosal cytokine responses were higher than systemic cytokine responses both for the serotypes 1 and 6., Table 2.

Cytokine imbalance: Cytokine imbalance were noted in serotype 1 vaccine at mucosal response only, Table 3.

Immune Efficacy: The immune efficacy of sertype 1 was 80% and for serotype 6 was 60%, Table 4. The efficacy percenatge difference is equal to 28.571%.Since practically good vaccine is that gaving around 90% protection and the efficacy percetage difference approaching one third .Thus serotype C 1 is more immune protective than serotype 6 in local multivalent candidate pneumococcal vaccine taking in consideration the limits of immune interference.

Prototype Carbohydrate Based Vaccine Features: *S. pneumoniae* serotype 1 prototype

vaccine was found; Pure, safe, antigenic, weak immunogenic needs exogenous adjuvant, induce humoral systemic precipitin responses higher than mucosal responses. Mucosal Cytokine responses were higher than systemic responses. Induce cytokine imbalance at mucosal surfaces but not at systemic compartment. It was immune efficus to 80% in a lapin challenge model. Serotype 6 prototype vaccine was found; Pure, safe, antigenic, week immungenic need exogenous adjuvant. Induce systemic humoral immune resoponses higher than mucosal responses. Mucosal cytokine responses were higher than the systemic responses. It neither induce cytokine imbalance at mucosa nor at systemic compartment. It was immune efficus to 60% in lapin challenge model, Table 5.

Table 1. Specific immune precipitins respnses of the immne challenged rabbits

Groups	Titres
Serotype 1 specific immune primed rabbits	
Mucosal response	20
Systemic response	400
Serotype 6 specific immune primed rabbits	
Mucosal response	13.33
Systemic response	213.33
Control rabbits	
Mucosal response	3.33
Systemic response	6.3

Table 2. Cytokine responses of immune primed challenged rabbits

Rabbit groups	TNF alpha	TNF beta	IL6	IL10
Serotype 1 specific immune primed challnged rabbits				
Mucosa	473.11-+29	387.21 -+1.39	360.59-+4.3	253.36-+23.5
Systemic	250-+30.7	250.11-+7.6	223.5-+20.2	202.6 -+14.5
Serotype 6 specific immune primed challenged rabbits				
Mucosa	233.55-+1.4	225.25-+7.3	238.8-+4.9	199.68-+13.5
Systemic	245.2-+35	236.32-+27.8	219.51-+59.6	200.76-+8.1
Control				
Mucosa	125.12-+55	110.35 -+11.5	93.54 -+2.5	110.25 -+ 11.5
Systemic	115.29-+11.7	101 -+11.5	100.62-+0.75	101.15 -+11.5

Table 3. *S. pneumoniae* capsular 1 and 6 primed challenged rabbits cytokine imbalance

Rabbit groups	TNF alpha	TNF B	IL6	IL10
Sertype 1 specific immune primed challenged rabbits				
Mucosa	4:1*	3:1	3:1	2:1**
Systemic	2:1	2:1	2:1	2:1
Serotype 6 specific immune primed challenged rabbits	1.5:1	2:1	2:1	2:1
Mucosa systemic	1:1	1:1	2:1	1:1
Control Mucosa/ systemic	1:1	1:1	1:1	1:1

• The number of folds of cytokine concentrations for the test to control rabbits

*** Imbalance is the number of concentration folds for the proinflammatory to the anti-inflammatory cytokines.*

Table 4. The immune efficacy of the developed vaccines in rabbits

Prototype vaccine	Infective dose	Number of rabbits	Servive to total	Percentages
Type 1	1x10 to 6	10	8:10	80%
Type 6	1x10 to 6	10	6:10	60%
Control	1x10 to 6	10	0:10	0%

Table 5. Features of the Carbohydrate based *S. pneumoniae* serotype 1 and 6 monotypic prototype vaccines in rabbit challenge model

Features	Protype C1	Prototype C6	Approved pneumococcal Vaccine
Understanding Disease	Pneumonia	Pneumonia	Pneumonia
Understaning causal	*S. pneumoniae*	*S. pneumoniae*	*S. pneumoniae*
Virulence factor	Serotype 1	Serotype 6	23 serotypes
Purity	Pure	Pure	Pure
Safety	Safe	Safe	Safe
Antigenicity	Antigenic	Antigenic	Antigenic
Immunogenicity	Immungen*	Immunogen*	Immunogen*
Immune efficacy in rabbit	80%	60%	60%
Immune efficacy in man	?	?	

• *Amplified on combination with lanlin*

4. DISSCUSSION

The subunit bacterins of the bacterial pathogens when introduced to the body of a small mammal laboratory animals like rabbits, Tables 1 – 5, may triggers TH1, TH2 and /or B cells to grow, proliferate, expand and activated as an effector and/ or memory cells. As a consiquences, humoral mucosal and systemic antibody responses as well as cytokine network activations both for innate and adaptive cytokine types [19,20] The systemic humoral immune responses were higher than mucosal responses [16]. While the mucosal cytokine responses were higher than the systemic responses [4]. Cytokine imbalance was noted betwen pro and anti-inflammatory cytokines serotype 1 at mucosal compartment but not systemic. Serotype 6 does not express imbalance at both mucosal and systemic compartments [21]. The immune effcacy of the present prototype carbohydrate based capsular bacterins were ranging between 60- 80% [11,22]. The vaccinologic features of the prepared candidates capsular bacterins were; Antigenic, weak immunogenic need an exogenous adjuvant leading to antibody and cytokine responses. Such responses were both at mucosal and systemic compartments with an immune efficacy ranging betwen 60 to 80% in rabbit models. The proposed *S. pneumoniae* vaccine strains may be of help in development of an eight local pneumococcal vaccine with the other reported six serotypes [10] which may be more efficieous in this area than the internationally approved 23 pneumococcal vaccine [22]. The vaccine strains form an integral part in vaccine development, since it forms the first step in vaccine development processes. S. pneumoniae serotype 1 and 6 are among the known serotypes ensembled in various up date and approve vaccine formulations [23,24].

5. CONCLUSION

Local pneumotropic isolates of *S. pneumoniae* serotype 1 and 6 were found valid as vaccine strains based on lapin immune challenged models. The proved immune characteristics of these capsule serotypes were suggestive for use of these strain as vaccine strains along with other serotypes in development of the local prototype multiserotype vaccines of this pathogen.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technology Models (Chat GPT, COILOT, etc) and text-to-image generator have been used during writing or editing of this manuscript.

CONSENT

It's not applicable.

ETHICAL APPROVAL

Care, housing, handling and managment done on rabbits were following the international acts regulating care, housing, handling and intervensions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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