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Laboratory Development and Shared Antigenicity of Two Prototype Candidate Citrobacter Freundii Vaccines

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Abstract

Cirobacter freundii are currently standing as a newly emerged human pathogen causing several infection types .It may take an outbreak pattern of nosocomial infectious disease. Thus, a prototype candidate Citrobacter freundii human uro-pathogen were aimed to be laboratory developed in two versions .The first was heat killed intact[CFKV] and the second was stealth live cell wall defective[CFSLV] vaccines. Both versions were found to be; safe, immunogenic and effective on lapin immune and challenge models. The immune efficacy was up to 80% for CF SLV and 60% for the CFKV vaccines. CFKV and CFSLV vaccines were with no mortality but with mild short lasting morbidity. The criteria of the laboratory developed C. fruendii vaccines were matching that of typhoid vaccine Ty21a.The stealth FCSLV of freundii vaccine has shown shared antigenicity with CFKV vaccine version. Such shared antigenicity was found to be of bilateral nature both in quantitative and qualitative terms. CFKV and CFSLV vaccines induced humoral antibody responses which may be of Th2 dependent B cell responses. T cell responses are far from being operable in the immune efficacy of these vaccines, since C .fruendii are neither obligate nor facultative intracellular parasites . Vaccine lapin cross challenge models are waiting to be explored in a future work.

Keywords: Antibody; Antisera; Shared antigenicit; Agglutinin; Absorption.

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1. Introduction

The intact cell walled and the stealth cell wall defective Citrobacter freundii are evident emerging opportunistic pathogen in general and uro-pathogen in particular. Their infection modes were as drug sensitive and multidrug resistant episodes [1,2,3,4].Hence, the interested professionals were attracted to develop C. freundii exo-polysaccharide vaccine[5],cell free culture filtrate CFC vaccine[6,7].The objective of the present communication was at: Developing a prototype candidate vaccines in two versions. The intact heat killed CFKV and the stealth cell wall defective CFSL , and ii – Studying the possible sharing antigenicity of these two vaccine versions using agglutinin absorption studies.

2. Material and Methods

1.1 Vaccine Strain

The vaccine strain of C .freundii was an opportunistic human uro-pathogen as confirmed by classical andAPI20E[8] .

1.2 Laboratory Development

Seed lot was prepared from a revived strain on brain heart infusion broth then onto brain heart infusion agar. Five identical colony morpho-type were transferred to nutrient broth and incubated for two hours at 37C. A 0.1 ml from the two hours culture was used to seed the 50 ml medium in a flask and incubated at 37C for an overnight period .The vaccine bacteria were checked for viability ,purity and found viable and pure .Then harvested by centrifugation at

4000 rpm for five mints . The dosing was rated up to1.5x10 to eight CFU/ml for CFKV. The final lot of the vaccine was dispensed at 2 ml. amounts and kept at4C for short term experimentation. Growth in bulk for stealth vaccine was made into high sucrose growth medium in 50 ml .,flask containing Impinim for 10 days at 37C then harvested by centrifugation at 5000 rpm for five mints and reconstituted by sterile saline to the original cell density, and the dose matched to 10 IU/ml. ,by standard WHO Opacimeter and made ready to specific immune priming of the Experimental rabbits[8,9] as in the followings;

Table 1: Animal Assignment

Group	Vaccine	Animals
Group I	CFKV	5 rabbits
Group II	CFKV	5 rabbits
Group III	saline	5 rabbits

The test rabbits were adapted to the housing conditions for two weeks, checked for the presence of common pathogen antibodies in their sera and proved to be negative. The specific immune priming of these rabbit groups was the multisite multi injection protocol[10] ,using 1x10 to seven CFU/ml., for CFKV and 1o IU/ml., for

CFSLV live infectious doses.

Table 2: The specific immune priming program of rabbits

Priming Time Table[10]	Dose	Dosing Pattern
First Week	2x 10 IU/ml.	0.25 ml. SC in
First day	,one ml.	each of the
Second day	1x10 IU/ml.	four* para-
	,one ml.	nodular areas
Second week	2x10 IU/ml.,	0.25 ml. SC in
First day	one ml.	each of the
Second day	1x10 IU/ml.	four para-
	,one ml.	nodular areas
Third week	1x10 IU /ml.,	0.25 ml. SC in
First day	one ml.	each of the
Second day	1x10 IU/ml.,	four para-
	one ml.	nodular areas
Fourth week	1x10 IU /ml.,	0.25 ml. SC in
First day	one ml.	each of the
Second day	1x10 IU / ml.,	four para-
Fifth week: Leave.	one ml.	nodular areas
Sixth week: test bled		

*Left and right sub-clavian shoulder region; Left and right pelvic region.

SC= Subcutaneous

1.3 Viability And Purity

The viability checked by direct microscopy to watch motile rod phenotype and by plate viable count. Purity also checked by microscopic detection of contaminant phenotype in addition to quadrate streak culture to scan contaminating colony morpho-types.

1.4 Immunogenicity

The immunogenicity of the vaccines was checked through agglutination studies of vaccine and lapin immune sera[10,11,12].

1.5 Efficacy

The efficacy in the lapin challenge model was measured as ;vitality, morbidity and mortality percentages of the challenged rabbits(12).

1.6 Safety

Gross signs of primed morbid animals for toxic unsafe changes observed on evisceration ,if any, will be checked histologically[11,12].

1.7 Shared Antigenicity

Shared antigenicity was detected using agglutinin cross-absorption studies[13,14,15].

3. Results

3.1 Laboratory Development

The in-vitro developmental evaluations criteria have shown that the vaccine bacteria in both vaccine versions were viable and pure. The in-vivo evaluation criteria were showing that the proto-type candidate vaccines were ;pure ,safe, immunogenic and effective in lapin challenge models. The immunogenicity of the CFKV vaccine was to the titre of 11946 While the immunogenicity of CFSLV vaccine was to the titre of 16936.The efficacy was up to 80% in case of CFSLV vaccine and of 60% for the CFKV vaccine .The two prototype vaccine versions are compared to the laboratory developmental criteria of Ty21a vaccine of Salmonella typhi ,Tables 3, 4 and 5.

Table 3: The immunogenicity of the two C. fruendii vaccine versions

Immune-sera/vaccine antigens	Titre*
Polyclonal anti-CFKV	11946
Polyclonal anti-CFSLV	16936

*Mean of five readings

Table 4: The immune efficacy of the two C.fruendii prototype vaccine versions

Immune-sera/vaccine antigens	Vitality	Morbidity	Mortality
Intact heat killed CFKV	3:5*(60%)	2:5(40%).	0:5(0%)
Stealth Cell wall defective CFSLV	4:5(80 %)	1:5(20%)	0:5(0%)

*Number of test rabbits

Table 5: The developmental criteria of C .freundii prototype vaccines

Immune-sera/vaccine antigens	Stealth CFSV	Intact CFKV	Ty21a*
Understanding the disease	Understandable	Understandable	Understandable
Understanding the causal	Understandable	Understandable	Understandable
Preparing prototype candidate vaccine and laboratory preclinical evaluations	Safe	Safe	Safe
Safety	Ratified	Ratified	Ratified
Dosing	Non-viable	Nonviable	Live attenuated
Viability	Pure	Pure	Pure
Purity	Immunogenic	Immunogenic	Immunogenic
Immunogenicity	Effective to	Effective to	Effective to
Efficacy	80%	60%	90%

*Based on [16].

3.2 Shared Antigenicity

The polyclonal unabsorbed antiserum ASIII when reacted with the antigen AGI gave a mean titre of 2139 but when reacted with AGII gave a mean titre of 1523.Homologous antigen-antibody reaction showed higher agglutinin titres than the heterologous antigen- antibody reactions. The absorption, and the reciprocal cross-absorption studies have shown that the ASV when reacted with the vaccine AGII gave a mean titre of 226,while when reacted with vaccine AGI gave a mean titre of 13 while when ASII reacted with AGI it also gave mean titre of 13.Absorption of polyvalent antisera with their homologous vaccine reduce but not diminish the antibody titres. Reciprocal cross-absorption of the polyclonal antisera revealed low titres with homologous vaccines and near diminished with heterologous vaccines, Tables 6 and 7.The results indicated that there were shared antigenicity between the two vaccine versions of C .fruendii, in a bilateral manner and both in quantitative and qualitative terms.

Table 6: Designations of Antisera and Vaccines

Entity	Description	Nature	Designation
Antiserum	Polyclonal anti-intact	Non-absorbed	ASI
	Polyclonal anti-intact	Absorbed with stealth vaccine	ASII
	Polyclonal anti-intact	Absorbed with intact	ASIII
	Polyclonal anti-stealth	Non-absorbed	ASIV
	Polyclonal anti-stealth	Absorbed with stealth	ASV
	Polyclonal anti-stealth	Absorbed with intact	AS VI
Vaccines	Intact heat killed		AGI
	Stealth cell wall defective		AGII

Table 7: Agglutination Assays of the vaccine versions and their antisera ,showing the shared antigenicity

Reaction	Assay Type Anti-Intact-Intact CFKV	Titre	Assay Type Anti-stealth-Stealth CFSLV	Titre
Reaction	ASI+AGI	11946	ASIV +AGII	16963
Cross reaction	ASI+AGII	1523	ASIV+ AGI	2139
Absorption	ASII+AGII	46	ASV+AGII	226
Cross-Absorption	ASIII+AGI	13	ASVI+AGI	13

4. Discussion

C. freundii as a human opportunistic uro-pathogen have several virulence factors like ;fimbrae ,toxins ,outer-membrane proteins, porins, lipoproteins and lipopolysaccharides [17].They are involved in 14-18% of clinical human cystitis cases[18] . It has been reported that C.freundii are currently emerging human uropathogen in an opportunistic mode of infection and it may took outbreak episode forms(1,2,3,4.19].The aforementioned situation forms good initiative for development of C. freundii vaccines[5,19].

Both of CFKV and CFSLV prototype vaccines were found pure, safe and immunogenic in rabbits models as well as they are with efficacy limits between 60 to 80 as lapin challenge model experiment have shown, the author in [5] have shown 90% efficacy in murine model using exo-polysaccharide vaccine .They express no mortality and mild urethral morbidity. The gained immunity from CFKV,CFSLV immunization in rabbits may be mediated by TH2-B lymphocyte cell-cell activation leading to humoral antibody responses rather than Th1 mediated immunity . Since C .freundii are neither obligate nor facultative intracellular pathogen[19]. Shared antigenicity of CFKV and CFSLV vaccines were found to be of major quantitative and minor qualitative nature as reciprocal cross-agglutinin assays indicated using vaccine primed lapin immunesera and prototype vaccine candidate antigens[20,21]. Stealth vaccine epitope(s) was potent agglutinin absorbers than that of intact vaccine epitope(s)[22].This difference may be attributed to their higher affinities of reaction with the available paratope in the prepared lapin sera[23]. This lapin challenge model was of promising results concerning the laboratory development of these two prototype vaccine versions of C. freundii ,but need to be confirmed in other nonhuman primate model before the initiation of clinical development. Since, the immune response to the vaccines in nonhuman vertebrate(lapin model) are different from that of human being immune response[24].

5. Conclusion

These two candidate C .freundii vaccines were proved to be pure, safe ,immunogenic ,and of 60 to 80% efficacy in lapin models but they are still in need for proving their development in lapin cross –challenge model and in non-human primate model.

6. Recommendation

The authors are of the opinion that the next step is to put-forward the laboratory development of these prototype vaccines in non-human primate model.

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