International Journal of Scientific Research in Biological Sciences
Research Paper
Vol.7, Issue.2, pp.44-50, April (2020)
E-ISSN: 2347-7520

Developement of anti- *P. aeruginosa* Immunoglobulin Y antibodies as prophylacic therapy for cystic fibrosis patients

Manal M. E. Ahmed [1,*], Jakeen Eljakee [2], Tarek Mahran [3]

[1]Pharmacology Department, Medical Research Division, National Research Centre, Egypt
[2]Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt
[3]Microbiology Department, Egyptian Atomic Energy Authority (EAEA), Egypt

[*]*Corresponding Author: thinktankteam2014@gmail.com, Tel.: +20-10936-27027*

Available online at: www.isroset.org

Received: 08/Mar/2020, Accepted: 12/April/ 2020, Online: 30/April/2020

Abstract— Emergence of multidrug-resistance (MDR) *P. aeruginosa* for respiratory tract infections represents a major critical challenge to the health care community worldwide. It is associated with progressive chronic respiratory infection in patients with cystic fibrosis (CF). While, the antibiotic resistance has been a global growing problem and vaccines development is impeded by several limitations, an urgent need to develop novel approaches is growing. In this study, we developed anti- *P. aeruginosa* IgYs as an alternative to meet this need. Preclinical evaluation revealed that immunization of Balb/c mice with the developed IgYs resulted in 95%, 90%, 70% protective effect among group (A) that was immunized intranasal one hour before challenge, group (B) that was immunized intranasal six hours after challenge and group (C) that was immunized orally, respectively. Furthermore, the pulmonary bacterial load in treated mice was lower than in the controls by more than 2 log after 24 hours of infection. Thus, the prepared IgYs had anti-*P. aeruginosa* immuno-reactivity that increasing its potential for use as a prophylactic therapy.

Keywords— P. aeruginosa, Immunoglobulins Y, Cystic fibrosis, protective efficacy, challenge test, prophylactic therapy

I. INTRODUCTION

Pseudomonas aeruginosa is an environmentally ubiquitous, gram-negative, opportunistic pathogen. It is commonly associated with progressive chronic respiratory tract infection in patients with cystic fibrosis (CF) and other causes of airway derangement [1]. Once colonization of the airways is established, *P. aeruginosa* is rarely eliminated despite an exuberant host inflammatory response [2]. The treatment of *P. aeruginosa* infection by an antibiotic therapy is limited due to a high incidence of drug resistance and the inability to completely eradicate infection in CF patients. Bacterial virulence factors as well as CF-specific host factors may play a role in the persistence of this organism [3-4]. Therefore, emergence of multidrug-resistance (MDR) *P. aeruginosa* for respiratory tract infections represents a major critical challenge to the health care community worldwide.

Antibiotics are an important tool in managing infections caused by MDR *P. aeruginosa* but the antibiotic resistance has been a global growing problem. Although vaccination can prevent infections, vaccines development is impeded by several limitations such as antigenic variations between strains, low efficacy or short-term immune responses [5-6]. In addition to rapid vaccine development may not be possible during outbreaks to control the spread of infection. So, despite considerable effort, vaccines against *P. aeruginosa* infection involving conventional immunization strategies have not been efficacious although recent novel approaches show some promise. The lack of progress toward the development of a vaccine against *P. aeruginosa* infection may be due to an incomplete understanding of the optimal *P. aeruginosa* antigens for the vaccine, as well as of the host immune mechanisms that mediate protective immunity against this pathogen [7].

Therefore, an urgent need to develop novel approaches to overcome *P. aeruginosa* infection. Passive immunization represents a possible alternative to face this need, especially in immune-compromised individuals as it avoids any side effects that might result from vaccination. Antibody preparations used for passive immunization mainly contain polyclonal antibodies derived from the sera of immunized animals, immunized humans, and in some cases convalescing patients. The effective use of polyclonal antibodies faces several challenges, including standardization and patient safety [8-9]. More recently, monoclonal antibodies (mAbs) have been suggested as an alternative to polyclonal antibodies, but their use is still very limited because of its high production cost. In addition, the possibility of *P.aeruginosa* escape mutants requires development of numerous mAbs targeting several antigens. This strategy would also increase the cost; time and effort as it require additional efficacy and safety studies [10-11].

Immunoglobulin Y antibodies (IgYs) are produced by chickens and other birds. IgYs are present in the sera of chickens and are passed to the embryo through egg yolk. IgYs containing dietary egg products have previously been

used against bacterial and viral infections in the human and animals. IgYs functions are similar to those of mammalian IgGs while IgYs have superior advantages over mammalian IgGs including lack of reaction with mammalian Fc receptors, low production cost, and ease of extraction [12]. In addition to IgYs have higher target specificity and greater binding avidity. They also possess remarkable pathogen-neutralizing activity in the respiratory tract. Furthermore, IgY is well tolerated because chicken eggs are a natural part of the human diet. It can be used in patients with egg allergies because the purified IgY does not contain egg albumin [13]. In some cases, passive immunization using IgY antibodies has rapid and local onset of action and can be given to patients with active infection or with impaired immune response [14]. An additional advantage of IgYs is their high content of sialic acid which is reported to increase the half-life of the drug compared with those with lower sialic acid content. This finding suggests that IgY-based therapy could have a longer circulating half-life, which could increase its efficacy against infections [15]. The aim of this work is to develop anti- *P. aeruginosa* IgYs for the most common *P. aeruginosa* strains in the field, followed by extraction and purification of the prepared anti-*P. aeruginosa* IgYs and evaluate their potential therapeutic applications for the prevention and treatment of *P. aeruginosa* infection.

II. METHODOLOGY

Bacterial strains: *P. aeruginosa* standard strain PAO1 was purchased from ATCC (Manassas, VA, USA). Clinical strains of six *P. aeruginosa* isolates were collected from Eldemerdash Surgery Hospital, Ain Shams University, Egypt (Table 1). Bacterial strains were cultured in Luria–Bertani (LB), washed and diluted with sterile PBS to an appropriate cell concentration determined spectrophotometrically at 600 nm (OD_{600}).

Strain	Lanyi-Bergans O-serogroups	No of isolates
170001	O3	3
170010	O6	4
170022	O15	1
170023	O12	1
170021	O4	2

Experimental Animals: Ten Single Comb White Leghorns SPF hens (6 months old) were purchased from a local poultry farm. The hens were kept in a standard poultry house with a 16/8 h light/dark cycle. Room temperature was approximately 25 °C. The hens were fed a laying hen diet, crushed egg shells, and water ad libitum.

Immunogen preparation: Cultured *P. aeruginosa* isolates were collected and suspended with Luria–Bertani (LB) to a concentration of 10^8 CFU/ml. One week before vaccination, aliquots of *P. aeruginosa* isolates were exposed to 3.6 K Gy irradiation. The inability of the irradiated bacteria to replicate was confirmed by plating on LB agar after incubating in a bacteria culture incubator for at least three days. The prepared aliquots of vaccine were then stored at - 20 °C before vaccination.

Anti-*P. aeruginosa* IgY preparation

Chicken immunization: After keeping for three days, 6 months old Single Comb White Leghorns SPF laying hens were vaccinated by 1 ml containing 1×10^8 CFUs of the prepared polyvalent irradiated *P. aeruginosa* immunogen intramuscular. Each hen was injected at four different sites (250 ul per site) of the leg muscle. Hens were administered four doses at two week intervals. The prepared immunogen was administrated in *Complete Freund's Adjuvant* (CFA) for the first dose and in In*complete Freund's Adjuvant* (IFA) for the booster doses. Blood samples were collected at day (0) and every two weeks before administration of each dose according to the method described by Yokoyama et al., 1997[11] with some modifications.

Some eggs were collected before immunization and stored in the refrigerator at 4 °C. One month after vaccination program, the eggs laid were collected daily for 1 month after vaccination program, marked and stored at 4 °C.

Extraction and Purification of anti-*P. aeruginosa* IgY

a. **Extraction of anti-*P. aeruginosa* IgY:** IgY was extracted from collected eggs using the water solution method as described by Akita and Nakai, 1993[16] and Chalghoumi et al., 2009[17]. Two weeks after the final immunization, collected eggs were harvested and pooled. The egg yolk was collected separately and carefully from the albumin and yolk membrane. The yolk membrane was perforated for allowing the flow of yolk into a graduated cylinder singly without the membrane. The egg yolk was mixed with fresh distilled water in ratio (1:9). The pH of the mixture was adjusted to 4.0 with hydrogen chloride (HCl) and stored overnight at 4 °C. The aggregate lipoproteins of the yolk were separated by centrifugation at 10,000 *Xg* for 25 min at 4 °C. Colorless and translucent supernatants that contain IgY were transferred to a graduated cylinder. The volume was recorded.

b. Salt precipitation of anti-*P. aeruginosa* IgY[18]: It was performed using ammonium sulphate 40% in two steps. First, the diluted yolk was precipitated by 40% ammonium sulfate at 4 °C using magnetic stirrer for 2 hours. Then, centrifugation was done at a speed of 10,000 Xg for 15 min. The pellet was re-suspended in 0.01 *M* Tris-HCl (pH 8.0) to a volume equal to half of the supernatant. The sample was precipitated by 40% saturated ammonium sulfate again at 4 °C for 2 hours, and the pellet was dissolved in PBS, pH 7.4, and dialyzed against 10 m*M* phosphate buffer, pH 7.0, for 3 successive days.

c. Anti-*P. aeruginosa* IgY concentration analysis: The protein concentration of the purified IgY was determined from the absorbance at 280 nm of purified IgY diluted at 1:10 using 1.33 as the extinction coefficient. The formula for calculating IgY concentration was as follows:

$$\text{IgY concentration} = A_{(280)} \times 10/1.33 \text{ mg/ml.}$$

Anti-*P. aeruginosa* IgY antibody titer was evaluated using ELISA: A polystyrene plate (Costar, USA) was coated with 100 ul of *P. aeruginosa* standard strain PAO1 in carbonate–bicarbonate (0.05 M, pH 9.6) coating buffer overnight at 4 °C. The plate was washed with PBS-T (0.01 M PBS containing 0.05% Tween 20), and then appropriately diluted IgY was added. After incubating for 2 h at 37 °C, the wells were washed with PBS-T, and 100 ul of an HRP-conjugated goat anti-chicken IgG (Sigma, USA) was added. After incubating for an additional 2 h, the plate was washed with PBS-T. Then, 100 ul of the TMB substrate solution was added. After further incubation for 15 min, 2 M H2SO4 was added to stop the reaction. The absorbance was measured at 450 nm on a micro plate reader (Thermo, USA). When the OD sample/OD negative ratio was greater than 2:1, the maximum dilution multiple of the sample was determined as the IgY titer.

Evaluation of the protective efficacy:

a. **Preparation of *P. aeruginosa* inocula for In Vivo challenge experiment:** *P. aeruginosa* strain PAO1 were grown overnight at 37 °C in LB. The bacteria were diluted 1:100 in fresh medium and grown to an OD_{600} of 1.0. For intranasal challenge experiments, bacteria were washed using phosphate buffered saline (PBS) and diluted to the indicated concentration that were determined by plate counting.

b. **Challenge experiment:** Eighty Balb/c mice were challenged intranasally with 1×1^{07} CFUs o*f P. aeruginosa* strain PAO1 directly into each nostril. The treatment using anti *P. aeruginosa* IgYs containing dietary egg products was applied, as the following: Mice were divided equally into four groups, Group (A) was immunized by placing 20 µl of the prepared *P. aeruginosa* IgY into each nostril (40 µl containing 40 ug per mouse) one hour before challenge with *P. aeruginosa* strain PAO1, Group (B) was immunized by placing 20 µl of the prepared *P. aeruginosa* IgY into each nostril (40 µl containing 40 ug per mouse) six hours after challenge with *P. aeruginosa* strain PAO1, Group (C) was immunized orally with 10 ml containing 10 mg of anti *P. aeruginosa* IgY using oral tube and Group (D) was administrated 10 ml of tap water as a control. All the administrations were continued twice daily for 14 successive days.

Broncho-alveolar lavage fluid (BALF) was collected daily for quantitation of CFUs. Clinical signs of *P. aeruginosa* were recorded from day 1 to the day 14 after inoculation. At day 14, the presence of the challenge organism was investigated by harvesting the lung, liver, blood, and spleen for bacterial load enumeration. A protected mouse was defined as a mouse that showed no clinical signs, no gross lesions at postmortem and failed to yield any challenge organism on culture.

Statistical analysis:

All data were analyzed using GRAPHPAD PRISM software (GraphPad, San Diego, CA). Data were analyzed using ANOVA (multiple groups), and multiple comparisons between the groups were performed using Newman–Keuls method after ANOVA. Survival data was plotted using Kaplan–Meier curves and analyzed by the log-rank test. For measurements of bacterial CFUs, groups were compared using a non-parametric Mann–Whitney U-test. $P < 0.05$ was considered as statistically significant for all experiments. All values were presented as the mean ± SD, with the exception of bacterial counts, for which median values were designated.

III. RESULTS

Anti - *P. aeruginosa* Ig Y concentration: Two weeks post immunization program with the irradiated *P. aeruginosa* isolates, laying hens produced specific IgY. The concentration of IgY was nearly 8.38 mg/ml egg yolk, as determined by absorbance at 280 nm.

Anti - *P. aeruginosa* Ig Y purity:

Antibody concentrations in the purified IgY samples was ranged from 8.03 mg/ml to 8.19 mg/ml as measured by Bradford assay.

Anti – *P. aeruginosa* IgY antibody titer in egg yolk:

As resulted using ELISA, anti - *P. aeruginosa* IgY produced by the vaccinated hens increased over time. The specific IgY titer increased starting the second week after the first immunization, with the titer peaking at the eighth week. After fourteen weeks, the antibody titer declined slowly (*Figure 1*).

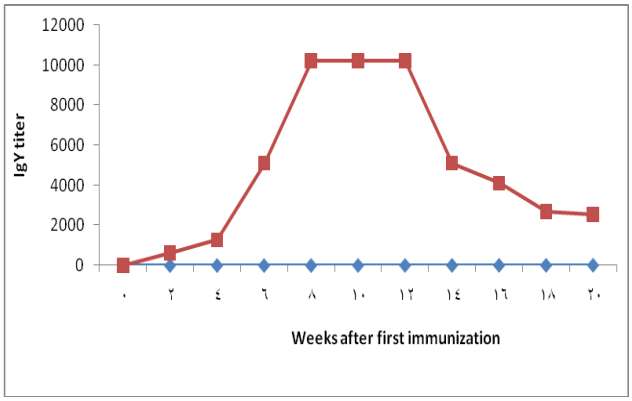

Figure (1): The titer of anti-*P. aeruginosa* IgY antibody in egg yolks increases over time. Egg yolks were collected from immunized hens over a course of 20 weeks after the first immunization.

Protective efficacy of the prepared anti- *P. aeruginosa* IgYs in vivo: We challenged eighty Balb/c mice intranasally with 1×10^7 CFUs of *P. aeruginosa* strain PAO1 directly into each nostril. Mice were divided into four equal groups, Group (A) was immunized by placing 20 µl of the prepared anti-*P. aeruginosa* IgYs into each nostril (40 µl containing 40 ug per mouse) one hour before challenge, Group (B) was immunized by placing 20 µl of the prepared anti-*P. aeruginosa* IgYs into each nostril (40 µl containing 40 ug per mouse) six hours after challenge, Group (C) was immunized orally with 10 ml containing 10 mg of anti-*P. aeruginosa* IgYs using oral tube and Group (D) was administrated 10 ml of tap water as a control. All the administrations were continued twice daily for 14 successive days. We found that the prepared anti-*P. aeruginosa* IgYs have significant protective and therapeutic effects against lethality of *P. aeruginosa* strain PAO1 ($p < 0.01$) when compared with the control group that was challenged by a relative. Immunization with anti-*P.aeruginosa* IgYs led to 95%, 90%, 70% protective effect among group (A), group (B) and group (C), respectively (Figure 2).

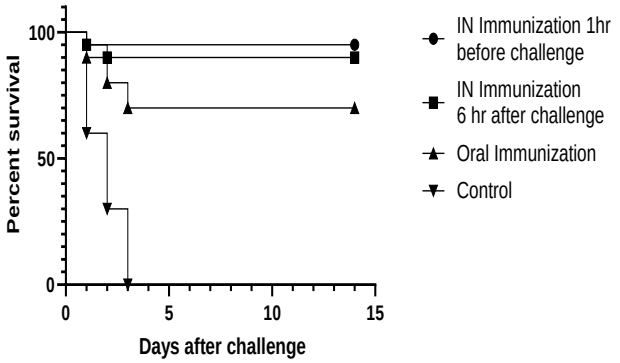

Figure (2): Kaplan–Meier curves were plotted for mice of the four groups which were challenged by 1×10^7 CFUs of *P. aeruginosa* strain PAO1 directly into each nostril, treated with anti-*P. aeruginosa* IgYs twice daily for 14 successive days and monitored the survival rates ($n = 20$, $P < 0.01$).

Clinical signs and gross lesions post challenge test:
Clinical signs of *P. aeruginosa* infection were recorded from day 1 to the day 14 after inoculation. The infected cases were suffering mainly from pneumonia with its characteristic clinical signs including fever and chills, difficulty breathing, cough which sometimes with yellow, green or bloody mucus. Fourteen days after inoculation, Died cases were subjected to postmortem inspection that revealed the presence of severe congestion in the internal organs including lung, liver and spleen. The presence of the challenge organism was investigated by harvesting the lung, liver, blood, and spleen for bacterial load enumeration. Numbers of cases per group those suffering from *P. aeruginosa* clinical signs and demonstrated colonization post challenge test are listed in Table (1).

Table (1): Numbers of cases per group were suffering from *P. aeruginosa* clinical signs and demonstrated colonization post challenge test.

Groups	No. of mice with clinical signs	Fate		With colonization
		Died	Recovered	
Group A	1	1	0	3
Group B	3	2	1	4
Group C	8	6	2	11
Group D	20	20	0	20

Quantitation of CFUs in Broncho-alveolar lavage fluid (BALF): Broncho-alveolar lavage fluid (BALF) was collected daily after challenge with 1×10^7 CFUs of *P. aeruginosa* strain PAO1 directly into each nostril. BALF was subjected for quantitation of CFUs. Titer of *P. aeruginosa* in BALF 24 hours post challenge test was as shown in Table (2).

Table (2): Titer of *P. aeruginosa* in BALF 24 hours post challenge test.

Vaccinated Groups	Colonization cases after 24 hours of infection			
	Number of cases	CFUs	Number of cases	CFUs
Group A	2	10^8	1	10^9
Group B	2	10^8	2	10^9
Group C	5	10^8	6	10^9
Group D	0	10^8	20	10^9

IV. DISCUSSION

Pseudomonas aeruginosa pulmonary infection is one of the life threatening infections. Its complications commonly cause bacteremia and sepsis in hospitalized and immunocompromised individuals [19-20]. The misuse of broad spectrum antibiotics empirically for everyday infections has led to the continuous emergence of multidrug resistant (MDR) *P. aeruginosa* strains that present a major challenge to clinical therapy that resulted in increased morbidity and mortality [21]. The high prevalence of infection with MDR *P. aeruginosa* strains accompanied by the paucity of new effective antibiotic classes present unique challenges to clinicians and highlight the need for developing new therapeutic approaches, such as immunotherapy, that targets *P. aeruginosa* virulence factors to reduce its pathogenesis without inducing resistance [22-23]. The complexity of *P. aeruginosa* genome which encodes numerous antigens indicates that immunotherapy depending on a single antigen will not provide effective protection.

Therefore, development of *P. aeruginosa* vaccines may be hindered by the complexity of *P. aeruginosa* genome. In addition, the numerous virulence factors of *P. aeruginosa* and its ability to infect different tissues have made it difficult to determine which virulence factors need to be targeted for effective immunity [24].

Passive immunization is the administration of preformed antibodies or immunoglobulins to treat specific infectious diseases [25]. Based on this principle, intranasal antibodies for prophylaxis were reported to demonstrate a promising approach for viral respiratory tract infections in experimental animals including respiratory syncytial virus (RSV), influenza virus, and Sendai virus. It is also expected to show sufficient protective efficacy in the clinical trials for influenza A and B viruses, Coxsackie virus, and rhinoviruses [26].

The developed IgYs has been approved for practice since 1996 [27]. The Veterinary Office of the Swiss Government also accepted IgYs practice in 1999[28]. For humans, IgY has been shown to be effective for prophylaxis and therapeutic uses of acne and other dermatological infections [29]. Also, it has been used for candidiasis, dental caries, and periodontitis [30-31]; gastritis and Helicobacter pylori [32-33]; intestinal disorders such as celiac disease, cholera, and diarrhea [34-35]; metabolic syndrome [36]; and illness caused by environmental factors, such as norovirus, dust mites, and snake venom [36-37].

Cystic Fibrosis is a multisystem autosomal recessive disorder affecting nearly 70.000 people worldwide. CF is mainly caused by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This CFTR dysfunction results in very thick secretions in the airways leading to difficult mucociliary clearance [39]. *P. aeruginosa* is a very common pathogen in CF patient resulting in a reduction in lung functions with high percent of morbidity and mortality. *P. aeruginosa* clearance is highly difficult and patients commonly experience chronic infection [40].

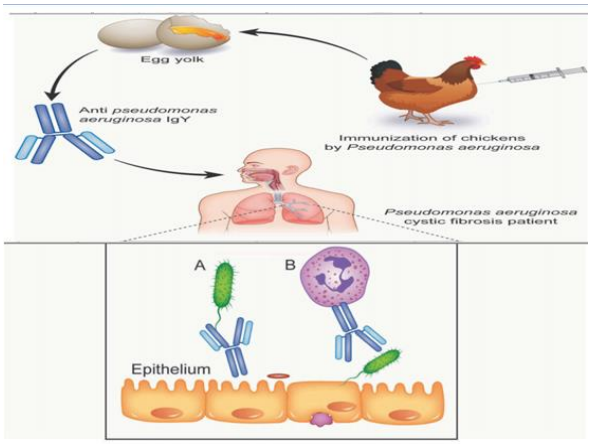

Figure (3): Preparation of anti–*P. aeruginosa* IgYs for prevention and treatment of *P. aeruginosa* in cystic fibrosis patients [38].

Binding of IgYs to *P. aeruginosa* early in the course of infection prevents adhesion of the bacteria to oropharynx which can prevent bacterial colonization [41].

In this study, we developed anti–*P. aeruginosa* IgYs against *P. aeruginosa* isolates O3, O4, O6, O12 and O15. Two weeks post immunization program with the irradiated *P. aeruginosa* isolates, IgYs were extracted from egg yolk. The concentration of IgY was nearly 8.38 mg/ml egg yolk, as determined by the absorbance at 280 nm. After purification steps, IgYs concentration was ranged from 8.03 mg/ml to 8.19 mg/ml as measured by Bradford assay. We used ELISA to determine specifically anti–*P. aeruginosa* IgY antibody titer. The specific IgY titer increased starting the second week after the first immunization, with the titer peaking at the eighth week. After fourteen weeks, the antibody titer declined slowly.

We evaluated the protective efficacy of the developed anti–*P. aeruginosa* IgYs in eighty Balb/c mice which were challenged intranasal with 1×10^7 CFUs of *P. aeruginosa* strain PAO1 directly into each nostril. Mice were administrated IgYs by different routes, Group (A) was immunized intranasal one hour before challenge, Group (B) was immunized intranasal six hours after challenge, Group (C) was immunized orally and Group (D) was administrated tap water as a control. All the administrations were continued twice daily for 14 successive days. Broncho-alveolar lavage fluid (BALF) was collected daily for quantitation of CFUs. Clinical signs of *P. aeruginosa* were recorded from day 1 to the day 14 after inoculation. At day 14, the presence of the challenge organism was investigated by harvesting the lung, liver, blood, and spleen for bacterial load enumeration. A protected mouse was defined as a mouse that showed no clinical signs, no gross lesions at postmortem and failed to yield any challenge organism on culture. The results revealed that the prepared anti-*P. aeruginosa* IgYs have significant protective and therapeutic effects against lethality of *P. aeruginosa* strain PAO1 ($p < 0.01$) when compared with the control group that was challenged by a relative. Immunization with anti-*P. aeruginosa* IgYs led to 95%, 90%, 70% protective effect among group (A), group (B) and group (C), respectively.
Mice administrated Intranasal 1hour before or 6 hours after infection, showed significant reduction of bacterial load than oral administration. Therefore, we can conclude that the prepared anti-*P. aeruginosa* IgYs decrease or prevent colonization as the prepared IgYs were found to induce rapid and competent bacterial clearance in vivo experiment.

In general, the pulmonary bacterial load in anti-*P. aeruginosa* IgYs treated mice was lower than in the controls by more than 2 log after 24 hours of infection. The IgYs were found to induce a rapid decline in the bacterial load within the first hours of infection. The IgYs treated mice had a better clinical state compared to controls, which may be attributable to a lack of disseminated infection since anti-*P. aeruginosa* IgYs protected against bacteremia. The prophylactic effects of intranasal administration of IgYs

were more notable than intranasal administration 6 hours after infection and oral administration, respectively. The superiority of the prophylactic treatment may be due to the presence of IgYs in the airways, which prepared the mucosal surface for the opsono-phagocytic process and reduced its interaction with the IgYs opsonized pathogens. The results revealed that the prepared IgYs had anti-*P. aeruginosa* immuno-reactivity increasing its potential for use as a prophylactic therapy but further studies are still needed to determine the levels of inflammatory cytokines with IgYs treatment. Also, IgYs should be tested in a large scale before introduced to human clinical trials [42-43].

V. CONCLUSION AND FUTURE SCOPE

We can conclude from this study that the prepared anti-*P. aeruginosa* IgYs provide protective efficacy ranged from 70% up to 95% according to the route of administration. Also, passive immunization using the prepared anti-*P.aeruginosa* IgYs led to decrease or prevent colonization as the prepared IgYs were found to induce rapid and competent bacterial clearance in vivo experiment. Thus, the promising anti-*P.aeruginosa* IgYs can be used as an effective prophylactic therapy against *P. aeruginosa* in cystic fibrosis patients.

VI. REFERENCES

[1] P.B. Davis, M. Drumm, M.W. Konstan, "Cystic fibrosis", Am. J. Respir. Crit. Care Med, Vol. 154, Issue. 5, pp. 1229–1256, 1996.

[2] P.H. Gilligan, "Microbiology of airway disease in patients with cystic fibrosis", J Clin Microbiol, Vol. 4, pp. 35–51, 1991.

[3] U.B. Schaad, A.B. Lang, J. Wedgwood, A. Ruedeberg, J.U. Que, E. Furer, S.J.J. Cryz, "Safety and immunogenicity of Pseudomonas aeruginosa conjugate A vaccine in cystic fibrosis", Lancet , Vol. 338, pp. 1236–1237, 1991.

[4] J.J. Smith, S.M. Travis, E.P. Greenberg, M.J. Welsh, "Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid", Cell, Vol. 85, pp. 229–236, 1996.

[5] J.L. McCaskill, S. Ressel, A. Alber, J. Redford, U.F. Power, J. Schwarze, B.M. Dutia, A.H. Buck, "Broad-spectrum inhibition of respiratory virus infection by MicroRNA mimics targeting p38 MAPK signaling", Mol Ther Nucl Acids, Vol. 7, pp. 256–266, 2017.

[6] D. Corti, N. Passini, A. Lanzavecchia, M. Zambon, "Rapid generation of a human monoclonal antibody to combat Middle East respiratory syndrome", J Infect Public Health , Vol. 9, pp. 231–235, 2016.

[7] A.W. Cripps, M.L. Dunkley, R.L. Clancy, J. Kyd, "Pulmonary immunity to Pseudomonas aeruginosa", Immunol Cell Biol, Vol. 73, pp. 418–424, 1995.

[8] M.A. Keller, E.R. Stiehm, "Passive immunity in prevention and treatment of infectious diseases", Clin Microbiol Rev, Vol. 13, pp. 602-614, 2000.

[9] B.S. Gaham, D.M. Ambrosino, "History of passive antibody administration for prevention and treatment of infectious diseases", Curr Opin HIV AIDS. Vol. 10, pp. 129–134, 2015.

[10] B. Kelley, "Industrialization of mAb production technology: the bioprocessing industry at a crossroads", mAbs, Vol. 1, pp. 443–452, 2009.

[11] A. Casadevall, E. Dadachova, L.A. Pirofski, "Passive antibody therapy for infectious diseases", Nat Rev Microbiol, Vol. 2, pp. 695–703, 2004.

[12] G.W. Warr, K.E. Magor, D.A. Higgins, "IgY: clues to the origins of modern antibodies", Immunol Today, Vol. 16, pp. 392–398, 1995.

[13] D. Carlander, J. Stalberg, A. Larsso, "Chicken antibodies: a clinical chemistry perspective", Ups J Med Sci, Vol. 104, pp. 179–189, 1999.

[14] K. Thomsen, L. Christophersen, T. Bjarnsholt, P.O. Jensen, C. Moser, N. Hoiby, "Anti-Pseudomonas aeruginosa IgY antibodies augment bacterial clearance in a murine pneumonia model", J Cyst Fibr, Vol. 15, pp. 171-178, 2016.

[15] H.H. Nguyen, T.M. Tumpey, H. J. Park, Y.H. Byun, L.D. Tran, V.D. Nguyen, P.E. Kilgore, C. Czerkinsky, J.M. Katz, B.L. Seong, J.M. Song, Y.B. Kim, H.T. Do, T. Nguyen, C.V. Nguyen, "Prophylactic and therapeutic efficacy of avian antibodies against Influenza virus H5N1 and H1N1 in mice", PLoS One, Vol. 5, pp. e10152, 2010.

[16] E.M. Akita, S. Nakai, "Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with an enterotoxigenic *E. coli* strain.", J Immunol Methods, Vol. 160, pp. 207-214, 1993.

[17] R. Chalghoumi, A. Thewis, Y. Beckers, C. Marcq, D. Portetelle, Y.J. Schneider, "Adhesion and growth inhibitory effect of chicken egg yolk antibody (IgY) on Salmonella enterica serovars Enteritidis and Typhimurium in vitro", Food borne Pathog Dis, Vol. 6, pp. 593-604, 2009.

[18] M. Kuroki, Y. Ikemori, H. Yokoyama, R.C. Peralta, F.C. Icatlo, Y. Kodama, "Passive protection against bovine rotavirus-induced diarrhea in murine model by specific immunoglobulins from chicken egg yolk.", Vet Microbiol, Vol. 37, pp. 135-146, 1993.

[19] P.H. Gilligan, "Infections in patients with cystic fibrosis: diag**no**stic microbiology update. Clinics in Laboratory Medicine", Vol. 34, Issue. 2, pp. 197–217, 2014.

[20] N. Safdar, C. Dezfulian, H.R. Collard, S. Saint, "Clinical and economic consequences of ventilator-associated pneumonia: a systematic review", Critical Care Medicine, Vol. 33, Issue. 10, pp. 2184–2193, 2005.

[21] B.K Chan., M. Sistrom, J.E. Wertz, K.E. Kortright, D. Narayan, P.E. Turner, "Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*", Scientific Reports , Vol. 6, pp. 26717, 2016.

[22] M.W. Douglas, K. Mulholland, V. Denyer, T. Gottlieb, "Multi-drug resistant Pseudomonas aeruginosa outbreak in a burns unit—an infection control study", Burns: Journal of the International Society for Burn. Injuries, Vol. 27, Issue. 2, pp. 131–135, 2001.

[23] M. Mudau , R. Jacobson, N. Minenza, L. Kuonza, V. Morris, H. Engelbrecht, M.P. Nicol, C. Bamford, "Outbreak of multidrug resistant Pseudomonas aeruginosa bloodstream infection in the haematology unit of a South African academic hospital", PLoS One, Vol. 8, Issue. 3, pp. e55985, 2013.

[24] G.P. Priebe, G.J. Meluleni, F.T. Coleman, J.B. Goldberg, G.B. Pier, "Protection against fatal *Pseudomonas aeruginosa* pneumonia in mice after nasal immunization with a live, attenuated aroA deletion mutant", Infect. Immun, Vol. 71, pp. 1453–1461, 2003.

[25] J. Kovacs-Nolan, Y. Mine. "Egg yolk antibodies for passive immunity", Annu Rev Food Sci Technol, Vol. 3, pp. 163–182, 2012.

[26] R. Weltzin, T.P. Monath, "Intranasal antibody prophylaxis for protection against viral disease", Clin Microbiol Rev, Vol. 12, pp. 383–393, 1999.

[27] R. Schade, A. Hlinak, "Egg yolk antibodies, state of the art and future prospects", Altex, Vol. 13, pp. 5–9, 1996.

[28] K. Selvan, R. Sentila, . A. Michael, "eneration and characterization of chicken egg yolk antibodies against propionibacterium acnes for the prevention of acne vulgaris", Indian J Dermatol, Vol. 57, pp. 15–19, 2012.

[29] El S.M. Ibrahim, A.K. Rahman, R. Isoda, K. Umeda, N. Van Sa, Y. Kodama", In vitro and in vivo effectiveness of egg yolk antibody against Candida albicans (anti-CA IgY)", Vaccine, Vol. 26, pp. 2073–2080, 2008.

[30] S.V. Nguyen, F.C. Jr. Icatlo, T. Nakano, E. Isogai, K. Hirose, H. Mizugai, M. Kobayashi-Sakamoto, H. Isogai, I. Chiba, "Anti-cell-associated glucosyl transferase immunoglobulin Y suppression of salivary mutans streptococci in healthy young adults", J Am Dent Assoc, Vol. 142, pp. 943–949, 2011.

[31] K. Yokoyama, N. Sugano, T. Shimada, R.A.K.M. Shofiqur, El. S.M. Ibrahim, R. Isoda, K. Umeda, N.V. Sa, Y. Kodama, K. Ito, "Effects of egg yolk antibody against Porphyromonas gingivalis gingipains in periodontitis patients", J Oral Sci, Vol. 49, pp. 201–206, 2007.

[32] H. Suzuki, S. Nomura , T. Masaoka, H. Goshima, N. Kamata, Y. Kodama, H. Ishii, M. Kitajima, K. Nomoto, T. Hibi, "Effect of dietary anti-Helicobacter pylori-urease immunoglobulin Y on Helicobacter pylori infection", Aliment Pharmacol Ther, Vol. 20, Issue. Suppl 1, pp. 185–192, 2004.

[33] K.S. Hong, M.R. Ki, H.M.A. Ullah, E.J. Lee, Y.D. Kim, M.J. Chung, A.K. Elfadl, J.K. Park, K.S. Jeong , "Preventive effect of anti-VacA egg yolk immunoglobulin (IgY) on Helicobacter pylori-infected mice", Vaccine, Vol. 36, pp. 371–380, 2018.

[34] N. Gujral, R. Lobenberg, M. Suresh, H. Sunwoo, "In-vitro and in-vivo binding activity of chicken egg yolk immunoglobulin Y (IgY) against gliadin in food matrix", J Agric Food Chem, Vol. 60, pp. 3166–3172, 2012.

[35] K. Hirai, H. Arimitsu, K. Umeda, K. Yokota, L. Shen, K. Ayada, Y. Kodama, T. Tsuji, Y. Hirai, K. Oguma, "Passive oral immunization by egg yolk immunoglobulin (IgY) to Vibrio cholerae effectively prevents cholera", Acta Med Okayama , Vol. 64, pp. 163–170, 2010.

[36] S. Rahman, K. Higo-Moriguchi, K.W. Htun, K. Taniguchi, F.C. Icatlo, T. Tsuji, Y. Kodama, S. Van Nguyen, K. Umeda, H. N. Oo, et al.,"Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients', Vaccine, Vol. 30, pp. 4661–4669, 2012.

[37] M. Hirose, T. Ando, R. Shofiqur, K. Umeda, Y. Kodama, S. Nguyen, T. Goto, M. Shimada, S. Nagaoka, "Anti-obesity activity of hen egg anti lipase immunoglobulin yolk, a novel pancreatic lipase inhibitor", Nutr Metab (Lond), Vol. 10, Issue. 1, pp. 70, 2013.

[38] A.T. Abbas, S.A. El-Kafrawy, S.S. Sohrab, E.I.A. Azhar, "EIA. IgY antibodies for the immunoprophylaxis and therapy of respiratory infections', Human Vaccines & Immunotherapeutics, Vol. 15, Issue. 1, pp. 264-275, 2019.

[39] J. Emerson, M. Rosenfeld, S. McNamara, B. Ramsey, R.L. Gibson, "*Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis", Pediatr Pulmonol, Vol. 34, pp. 91–100, 2002.

[40] G.M. Nixon, D.S. Armstrong, R. Carzino, J. B. Carlin, A. Olinsky , C.F. Robertson, K. Grimwood, "Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis", J Pediatr, Vol. 138, pp. 699–704, 2001.

[41] E. Nilsson, A. Larsson, H.V. Olesen, P.E. Wejaker, H. Kollberg, "Good effect of IgY against Pseudomonas aeruginosa infections in cystic fibrosis patients", Pediatr Pulmonol, Vol. 43, pp. 892–899, 2008.

[42] M.M.E. Ahmed, W. Nazmy, J. Eljakee, "Preparation and Evaluation of Novel Anti-Obesity Immunoglobulins for Immunoprophylaxis and Therapy", International Journal of Scientific Research in Biological Sciences, Vol.6, Issue.6, pp.81-88, (2019).

[43] A.M. Abdou, M.M.E. Ahmed, Y. Yamashita, M. Kim, "Immunoglobulin: A Natural Way to Suppress Helicobacter pylori in Humans", Health, Vol.6, pp.781-791, 2014.

AUTHORS PROFILE

Dr. Manal M. E. Ahmed is Immunologist, Researcher, Columnist, Author, Reviewer, Public Speaker, Consultant and Principal Investigator of one of the national challenge projects in Egypt. She studied at Cairo University where she successfully completed B.Sc., M.Sc. and Ph.D. in microbiology and immunology. She also studied Business Administration at Ain Shams University where she successfully completed MBA. Currently, working researcher at the Department of Pharmacology, Medical Research Division, National Research Centre, Egypt. She worked over almost 25 years in the field of preparation of vaccines, immunoglobulin Y, polyclonal antibodies, and monoclonal antibodies as immunological tools for pharmaceutical and diagnostic industries. She has been awarded two patents from the academy of scientific research, Egypt. She published different scientific researches and articles in the scope of her work. She also interested in providing a social awareness for her community through the multimedia activities. She is the principal investigator of one of the national challenge projects of her country. She was actively participated in many scientific national and international seminars, workshops, and conferences. Her main interest is the employ of Immunology in innovating novel biological products that have pharmaceutical and diagnostic applications.

