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B Cell Response After Intradermal Inoculation of Multidrug Resistant *Enterococcus Faecalis* in Swiss Albino Mice

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Abstract

As resistance to last-resort antibiotics, *Enterococcus faecalis* is emerging as a significant health risk. To combat this, immune prophylaxis can provide effective alternatives. This research aims to assess the protective efficacy of antibodies generated by inactivated vaccinations against multidrug-resistant *E. faecalis*. This study include the intradermal inoculation of formalin-inactivated MDR *E. faecalis*, isolated from urine samples, into 15 Swiss albino mice. Two weeks following the third immunization dose, mice were challenged intraperitoneally with live *E. faecalis* and monitored for 14 days. Tail blood samples were collected on the 14th day after each inoculation, followed by cardiac puncture post-challenge. Mice spleens were cultured in RPMI media with fetal bovine serum for six days, and supernatants were collected. The antigen-binding capacity of serum antibodies and splenic lymphocyte culture supernatants generated by the vaccine was evaluated using ELISA. The study found that 100% of the vaccinated mice survived the challenge. Both pre- and post-challenge immunized serum immunoglobulin G antibodies and splenic lymphocyte culture supernatants exhibited significantly higher optical density values in ELISA compared to control mice. Immunization with formaldehyde-inactivated *Enterococcus faecalis* resulted in increased levels of IgG antibodies in both serum and splenic lymphocyte culture supernatants, leading to a 100% survival rate in immunized mice. Therefore, it can be concluded that immunization of Swiss albino mice with formaldehyde-inactivated MDR *Enterococcus faecalis* generates antibodies and induces a B cell response.

Keywords: *Enterococcus faecalis*, Immunization, inactivated vaccine, protective immunoglobulin G.

Introduction

In the past decade, *Enterococci* have gained prominence in clinical environments due to their capacity to cause severe infections and their growing resistance to several antibiotic treatments. The worldwide spread of multidrug-resistant *Enterococci* has considerably diminished treatment options. A vaccine for *Enterococcus faecalis* could eliminate or reduce the reliance on antibiotics, addressing several issues associated with antibiotic resistance. Attention should focus on developing killed or live-attenuated vaccines to enhance the immune system's presentation of various antigens (1). The inactivation method could be utilized with an innovative development strategy to prevent drug-resistant infections (1). Formaldehyde, widely used in vaccine production, serves as an effective deactivation agent. This type of vaccine antigen affects the interactions among dendritic cells,

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B cells, and T cells within germinal centers, thus impacting the duration and strength of protective immunity (2). The presence of Langerhans cells, dendritic cells, and other antigen-presenting cells (APCs) in the dermal layers renders these areas easily accessible and highly immunologically relevant (3). APCs capture most antigens introduced to the skin. During inflammatory responses, these APCs process and present invading antigens before migrating to secondary lymphoid organs via dermal lymphatic vessels. This anatomical arrangement makes dermal tissue an excellent site for initiating immune responses (3).

The role of B-cells in effective immune responses to vaccines is uncontested. The most successful vaccines (such as tetanus and diphtheria toxoids) generate protective, long-lasting humoral immune responses. It is widely accepted that this enduring humoral immunity arises from long-lived plasma cells that produce antibodies and memory B-cells (MBCs) (4; 5). MBCs respond quickly and specifically to re-exposure to antigens, contributing to both transient and persistent plasma cell populations and thereby extending high antibody levels in serum (6; 7). Furthermore, MBCs can persist throughout a host's lifetime, thus aiding in the swift clearance of pathogens upon re-exposure (8; 9; 10). To date, no studies have evaluated the B cell response in mice following vaccination against *Enterococcus faecalis* in Bangladesh. Therefore, this study aims to investigate B cell responses after the intradermal immunization of formaldehyde-inactivated multidrug-resistant *Enterococcus faecalis* in a murine model.

Materials and Methods

This study was conducted between November 2022 and June 2023 at the Department of Microbiology, Dhaka Medical College. Urine samples were collected aseptically from clinically suspected urinary tract infected patients attending the inpatient and outpatient department of Dhaka Medical College Hospital.

Ethical obligations Informed written consent was obtained from all the participants during the enrolment in the study. Data were entered in an anonymized and de-identified manner prior to analysis. Ethical approval for this study was taken from the Ethical Review Committee (ERC) of Dhaka Medical College.

Bacterial Culture: A total of 450 urine samples were gathered from both inpatients and outpatients at Dhaka Medical College Hospital in Dhaka, Bangladesh. From these samples, 23 multidrug-resistant *Enterococcus faecalis* strains were isolated. Six of these MDR strains were selected for the preparation of a vaccine. All bacterial cultures were incubated at 37°C for 24 hours prior to use, ensuring the bacteria were at a consistent growth stage throughout the experiment.

Immunization of Mice: Fifteen Swiss albino female mice, aged 3 to 4 weeks, were sourced from the Animal Resources Facility of ICDDR'B and maintained in a specific pathogen-free setting within the animal house at Dhaka Medical College. The mice were randomly divided into three groups: Group 1 (experimental group), Group 2 (negative control), and Group 3 (healthy control), with five mice in each group. All mice were provided with non-medicated feed and water throughout the study. The animal study received approval from the ethical review committee of the corresponding institution.

Preparation of Formalin Inactivated *E. faecalis* Vaccine: Group 1 received a formalin-inactivated vaccine prepared from a mixed solution of *E. faecalis* isolated from urine samples. A loop of the bacteria was inoculated into tryptic soy broth (TSB) and incubated overnight at 37°C. After incubation, the supernatant was discarded following centrifugation at 2,000 g for 20 minutes at 4°C. The pelleted bacteria underwent two washings with phosphate-buffered saline (PBS). For the creation of formalin-inactivated *E. faecalis*, 37% formalin was added to the bacterial suspension to achieve a final concentration of 3% (v/v) and incubated at 37°C for 2 hours. Afterward, the solution was washed twice with sterile PBS and resuspended in PBS to reach a concentration of 1.5 X 10^8 CFU/ml. An inoculum consisting of 134 µl was mixed with 866 µl of sterile PBS to achieve a concentration of 2 X 10^7 CFU/ml.

Immunization Schedule: Mice were sedated prior to each inoculation using an intraperitoneal dose of ketamine calibrated to their body weight (100 mg/kg). Ketamine acted as a muscle relaxant, while chloroform maintained a stable anesthetic condition. On days 0, 14, and 28, the experimental group (Group 1) received three intradermal injections of 20 µl of the bacterial solution (2 x 10^7 CFU/ml). Concurrently, the negative control group (Group 2) was injected with 20 µl of PBS. The intradermal inoculations were performed using a BD Ultra Fine™ (31G) insulin syringe.

Blood Collection for ELISA: Tail blood was collected 14 days following each vaccination. The tail was stretched and cleaned with 70% alcohol. A sterile scalpel (22 FR) was used to make a 2-mm incision on the tail, allowing for the collection of 50 µl of fresh blood in a microcentrifuge tube containing 200 µl of PBS to maintain a dilution of 1:5. The diluted serum was kept upright for 2 hours before centrifugation at 13,500 g for 10 minutes, and the clear serum was then transferred to a separate microcentrifuge tube.

Intraperitoneal Challenge: Two weeks after the last immunization, Groups 1 and 2 were challenged intraperitoneally with a dose of 3 x 10^8 CFU/ml in 100 µl PBS. The strains used for the vaccine formulation were employed to create the inoculum. All mice were observed for

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clinical signs such as weight loss, reduced mobility, and lack of appetite for 14 days following the challenge.

Cardiac Puncture Blood Collection: Blood was collected through cardiac puncture from Groups 1 and 3, 14 days post-challenge. The mice's chests were shaved and thoroughly cleaned with povidone iodine and 70% alcohol. The heartbeat was located with a finger, and blood was drawn from the heart using an insulin syringe inserted at a 45° angle. One milliliter of blood was collected and stored undiluted in a sterile test tube. Serum was separated as previously described and stored at -20°C for later use.

Culturing Splenic Lymphocytes in RPMI Media: Spleens from Groups 1 and 3 were harvested under aseptic conditions to assess the presence of antigen-specific B cells. The spleen tissue was crushed between two frosted glass slides, and the cell suspension was filtered through a 70 µm nylon cell strainer. The cells were then centrifuged at 350 g for 10 minutes at 4°C. The supernatant was discarded, and the cell pellet was resuspended in 5 ml of complete RPMI medium. After determining cell viability, a 1 ml portion of the splenic cell suspension was placed in a 24-well culture plate. The culture was supplemented with 50 µl of diluted antigen (sonicated crude *E. faecalis*) and incubated at 37°C for 6 days. Following incubation, the culture supernatant was collected, centrifuged, and stored at -20°C for future analysis.

Antibody Detection by ELISA: The optical density (OD) of mouse sera and splenic lymphocyte culture supernatants was measured using ELISA to evaluate the presence of immunoglobulin G (IgG) specific to *E. faecalis* antigens.

Procedure for Sonication of *Enterococcus faecalis*: Bacterial pellets were resuspended in 100 µl of distilled water, incubated on ice for 30 minutes, and then sonicated at 20 kHz for 2 to 10 seconds depending on sample viscosity. The samples were kept on ice for an additional 5 minutes before centrifugation at 10,000 g for 20 minutes to pellet debris (which may have included unlysed cells, nuclei, or organelles). The supernatant was transferred to a new microcentrifuge tube and stored at -20°C for future use as an antigen. Checkerboard titration was performed to optimize the antigen concentration, and 10 µg of antigen was utilized in the ELISA procedure.

Optimized ELISA: ELISA plates were coated with 100 µl/well of antigen (10 µg/ml) in a bicarbonate coating buffer (pH 9.6). Following an overnight incubation at 4°C, plates were washed three times with PBS and subsequently blocked with 200 µl/well of 5% w/v skimmed milk in PBS for 2 hours at 37°C. After washing with PBS-Tween (0.05% Tween 20) and once with PBS, serum samples and splenic lymphocyte culture supernatants were added at various dilutions (100 µl/well) and incubated at 37°C for 1.5 hours, then at 4°C overnight. Following another round of washing, a diluted horseradish peroxidase-labeled anti-mouse IgG antibody (Thermo Fisher Scientific, USA) was added at a 1:5000 dilution in blocking buffer (100 µl/well) and incubated at 37°C for 90 minutes. After further washing, 100 µl/well of a substrate solution containing tetramethylbenzidine (50 µl) and urea peroxide (50 µl) was added and incubated for 15 minutes at 37°C. The reaction was halted by adding 50 µl of 1M sulfuric acid. Absorbance was then measured at 450 nm using an ELISA plate reader (BioTek Inc, USA). The cutoff value for OD was calculate using the formula: OD = M (mean) + 2 × standard deviation

Data Processing

The data were carefully collected and meticulously revised through thorough examination and reexamination. Any errors and inconsistencies were systematically corrected.

Data Analysis

The results of the study were documented scientifically. Data were assessed and compared using a T-test. Statistical analysis of all information was performed with SPSS version 25 by SPSS Inc. A p-value of 0.05 was considered the threshold for statistical significance.

Results

The survival rates of immunized and unimmunized mice following a lethal challenge were recorded. All mice in Group 1 (the experimental/vaccinated group) survived throughout the 14-day observation period after the challenge. In contrast, all mice in Group 2 (the negative control group) succumbed within 24 hours of the challenge.

Table 1 presents the optical density values of IgG absorbance (450 nm) across different inoculation schedules within the experimental group, as analyzed by a one-way ANOVA. The calculated F value (77.71851) exceeded the critical F value (3.238872) from the F distribution table, and the p-value was less than 0.0001, indicating statistically significant differences. Therefore, significant differences were observed in the serum IgG antibody titers of the experimental group of mice at various stages of the vaccine inoculation schedule.

Table 1: OD Value Differences in Immune Response Following Each Inoculation of Formalin-Inactivated Vaccine Analyzed by ANOVA

	Sum of square	Df	Mean of square	F value	F crit	P value
Between group	3.294954	3	1.098318	77.71851	3.238872	<0.0001
Within group	0.226112	16	0.014132			
Total	3.521066	19				

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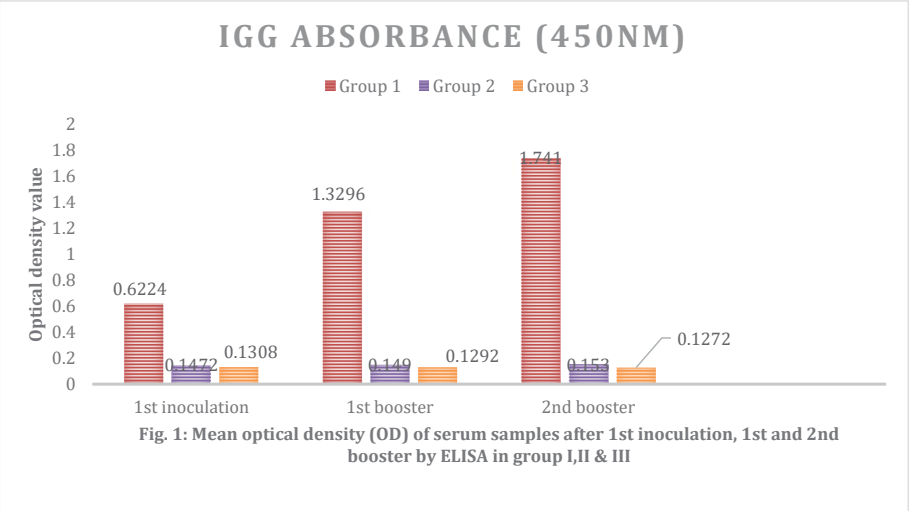

Fig. 1: Mean optical density (OD) of serum samples after 1st inoculation, 1st and 2nd booster by ELISA in group I,II & III

Figure 1: Illustrates the optical density of IgG antibodies in serum samples obtained 14 days after the first, second, and third inoculations. All serum samples from the vaccinated group (Group 1) exhibited optical densities of anti-*Enterococcus faecalis* IgG polyclonal antibodies exceeding the cutoff value. A statistically significant difference was observed between the optical density values of the experimental and control groups' sera, with a p-value of less than 0.0001.

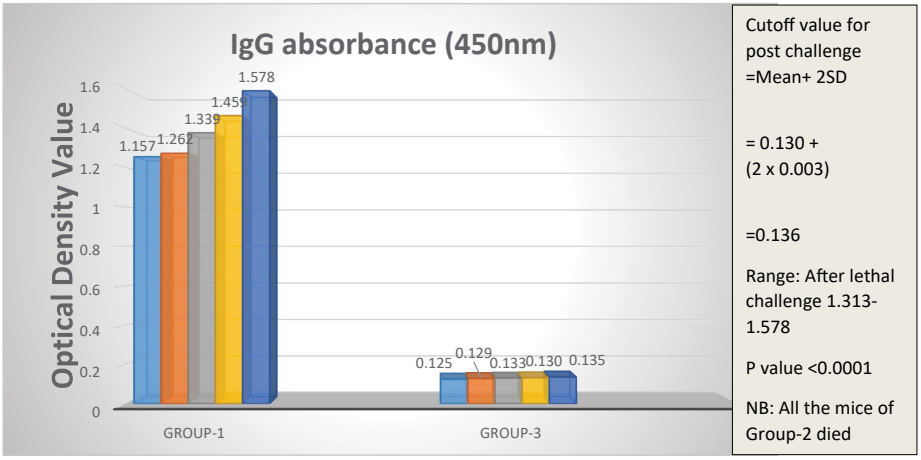

Figure 2: shows the optical density of IgG antibodies in serum samples collected after the lethal challenge. Five serum samples were taken from Group 1 mice, and all samples displayed optical densities of IgG polyclonal antibodies above the cutoff value of 0.136, which was calculated as the mean plus two standard deviations (2SD). The mean optical density values for the sera of the negative control mice were 0.130, with a standard deviation of 0.003. A statistically significant difference was observed between the optical density values of the experimental and control groups' sera, with a p-value of less than 0.0001.

Discussion

In this research, *Enterococcus faecalis* isolated from urine samples was inactivated using formaldehyde and subsequently used to immunize five Swiss albino mice in the experimental group (Group-1). Both the experimental group and a control group (Group-2) were subjected to lethal challenges. Remarkably, the vaccinated mice exhibited a survival rate of 100% at 14 days post-challenge. There is limited data on the survival rates of mice following lethal challenge after intradermal immunization with formalin-inactivated *E. faecalis*. However, a study conducted by 11 noted an 80% survival rate among vaccinated mice at 14 days post-challenge. Additionally, research from Korea indicated survival rates ranging from 80% to 100% (dependent on dose) at 5 days post-challenge after mice were immunized with *K. pneumoniae*-derived extracellular vesicles (12). In the current investigation, whole cell bacteria inactivated by formaldehyde were utilized to enhance the immune system's presentation of multiple antigens, thereby stimulating a more comprehensive immune response. The intradermal route was chosen due to the skin being the body's largest organ, containing a significant number of T lymphocytes and a

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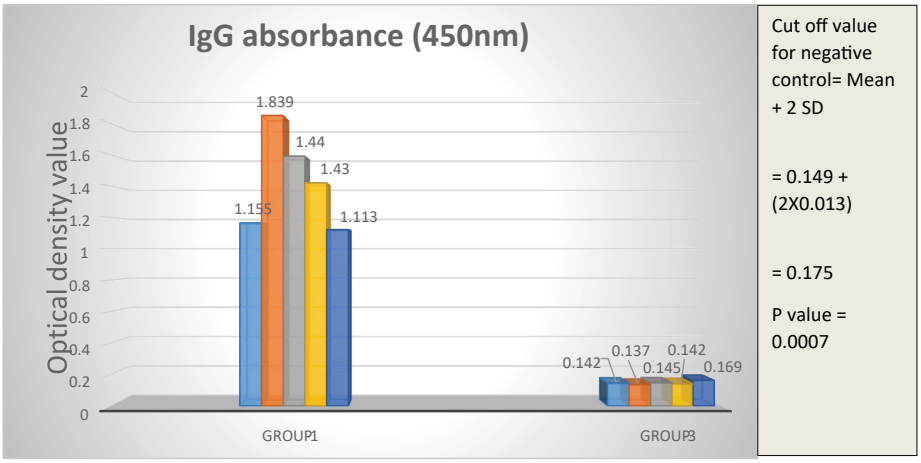

Figure 3: depicts the mean optical density of IgG antibodies in cell culture supernatants collected after culturing splenic cells in RPMI media. All samples from the experimental group demonstrated IgG polyclonal antibody optical densities exceeding the cutoff value of 0.167. The mean optical density of the cell culture supernatant from negative control mice was 0.147, with a standard deviation of 0.01. Statistically significant differences were noted in the optical density values between the experimental and negative control groups' cell culture supernatants, with a p-value of 0.007.

dense population of antigen-presenting cells (APCs) (13). Research by (11) in Bangladesh suggested that intradermal immunization is a promising method for inducing a high level of protective antibodies in experimental BALB/c mice. Another study by (14) in Bangladesh found that, although less effective than the intradermal route, oral administration of a formalin-inactivated whole cell vaccine also produced protective antibodies in vaccinated mice. Furthermore, (15) discovered that administering four doses of a plasmid containing the OmpK36 gene via intradermal injection in mice resulted in a mixed Th1/Th2 immune response and offered protection against lethal bacterial challenges.

In this study, serum samples were collected after each inoculation to analyze IgG antibody absorbance using ELISA at a wavelength of 450 nm. The results revealed a significant increase in the OD (optical density) (fig1) of serum IgG antibody absorbance within the experimental group following the first and second booster vaccinations. This increase could be attributed to enhanced production of IgG antibodies by B cells from the second booster onward. Following cardiac puncture, blood was collected from both the vaccinated group (Group-1) and the control group (Group-3). Subsequently, the spleen was extracted from the mice and cultured in RPMI media. Splenocytes were incubated in the media to invoke B-cell responses against sonicated antigens. A stimulation method was employed to promote the proliferation and differentiation of B cells into plasmablasts, leading to the secretion of antibodies, which were then detected in the culture supernatant (Fig3) . (16) Explored B-cell immune responses to *Vibrio cholerae* infection using a polyclonal stimulation method, which successfully enhanced the detection of B cells in circulation by inducing their proliferation and differentiation into antibody-secreting plasmablasts.

Conclusion

The prophylactic use of bacterial vaccines provides a valuable strategy for preventing bacterial infections. This approach may reduce the need for antibiotic prescriptions and alleviate the selective drug pressure that contributes to the emergence of resistant strains. While a definitive immunological correlate of protection for the formalin-inactivated *E. faecalis* vaccine has yet to be established, the findings of this study offer important insights for a novel strategy in vaccine development using formalin-inactivated bacteria, evaluated by ELISA, to protect against multidrug-resistant bacterial infections. Our results indicate that immunization with formalin-inactivated multidrug-resistant *E. faecalis* produces protective antibodies in Swiss albino mice.

Ethical Clearance

Ethical approval for this study was granted by the Institutional Review Board.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Fan Y, Mu Y, Lu L, et al. Hydrogen peroxide-inactivated bacteria induces potent humoral and cellular immune

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DOI:10.26502/ami.936500223

responses and releases nucleic acids. *International Immunopharmacology* 69 (2019): 389-397.

2. Amanna IJ, Carlson NE and Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. *New England Journal of Medicine* 357 (2017): 1903-1915.

3. Levin C, Perrin H and Combadiere B. Tailored immunity by skin antigen-presenting cells. *Human vaccines & immunotherapeutics* 11 (2015): 27-36.

4. Tangye SG, Avery DT, Deenick EK et al. Intrinsic differences in the proliferation of naive and memory human B cells as a mechanism for enhanced secondary immune responses. *The Journal of Immunology* 170 (2003): 686-694.

5. Tangye SG and Tarlinton DM. Memory B cells: Effectors of long-lived immune responses. *European journal of immunology* 39 (2009): 2065-2075.

6. Pinna D, Corti D, Jarrossay D, et al. Clonal dissection of the human memory B-cell repertoire following

infection and vaccination. *European journal of immunology* 39 (2009): 1260-1270.

7. Tarlinton D, Radbruch A, Hiepe F et al. Plasma cell differentiation and survival. Current opinion in immunology 20 (2008): 162-169.

8. Kelly DF, Pollard AJ and Moxon ER. Immunological memory: the role of B cells in long-term protection against invasive bacterial pathogens. *Journal of American Medical Association* 294 (2005): 3019-3023.

9. Blanchard-Rohner G, Snape MD, John T, et al. The magnitude of the antibody and memory B cell responses during priming with a protein-polysaccharide conjugate vaccine in human infants is associated with the persistence of antibody and the intensity of booster response. *The Journal of Immunology* 180 (2008): 2165-2173.

10. Blanchard-Rohner G, Pulickal AS, Zijde CM, et al. Appearance of peripheral blood plasma cells and memory B cells in a primary and secondary immune response in humans. *Blood. The Journal of the American Society of Hematology* 114 (2009): 4998-5002.

11. Kawser Z and Shamsuzzaman SM. Intradermal Immunization with Heat-Killed Klebsiella pneumoniae leading to the Production of Protective Immunoglobulin G in BALB/c Mice. *International Journal of Applied and Basic Medical Research* 11 (2021): 160-166.

12. Lee WH, Choi HI, Hong SW, et al. Vaccination with Klebsiella pneumoniae-derived extracellular vesicles protects against bacteria-induced lethality via both humoral and cellular immunity. *Experimental & molecular medicine* 47 (2015): e183-e183.

13. Pulit-Penaloza JA, Esser ES, Vassilieva EV, et al. A protective role of murine langerin+ cells in immune responses to cutaneous vaccination with microneedle patches. *Scientific reports* 4 (2014): 6094.

14. Rahman A, Shamsuzzama SM, Dola NZ and Nabonee MA. Protective effects of immunization with formalin inactivated oral whole cell vaccine against multidrug resistance *Citobacter freundii* in murin model. Americal Journal of Infectious Disease and Microbiology 10 (2022): 92-97

15. Kurupati, P, Ramachandran NP and Poh CL. Protective efficacy of DNA vaccines encoding outer membrane protein A and OmpK36 of Klebsiella pneumoniae in mice. *Clinical and Vaccine Immunology* 18 (2021): 82-88.

16. Harris AM, Bhuiyan MS, Chowdhury F, Khan AI, et al. Antigen-specific memory B-cell responses to Vibrio cholerae O1 infection in Bangladesh. *Infection and immunity* 77 (2009): 3850-3856.

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