

Review Article

STRATEGIES FOR DEVELOPING VACCINE AGAINST AVIAN PATHOGENIC *ESCHERICHIA COLI*

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Abstract

Avian colibacillosis is the most common bacterial infection in poultry industry world-wide. Prevention and control is challenging specially small and medium sized poultry farms. This article provides some insights regarding prevention and control of colibacillosis in poultry and special focus on strategies that scientist are adopting for developing vaccines against Avian Pathogenic Escherichia coli (APEC). As APEC has several serotypes, major are O1, O2 and O78. Live-attenuated vaccines are primarily based on these serotypes. During recent years, bacterial ghost platform system which utilizes the E-lysis gene from bacteriophage PhiX247 and staphylococcal nuclease A is being extensively studied for its possibilities of using as potential vaccine candidate. Very specific and robust subunit vaccine candidates are being studied and many candidates are in research pipeline to prepare recombinant protein and study their potency as vaccine candidates.

Key words: APEC, avian colibacillosis, vaccine, recombinant protein, bacteria ghost, serotypes

1 Background

Colibacillosis caused most common bacterial pathogen in poultry. It is one of the major bacterial threat in poorly managed small holder poultry farming system like in Nepal. Contaminated water and feed are the major source of APEC in poultry, however, fecal contamination of incubated eggs, transovarian contamination is also prevailing in poorly managed hatcheries (Giovanardi et al., 2005). Economic losses due to mortality, poor growth performance, and cost incurred in treatment & control of disease is of major concern. The clinical stage of colibacillosis depends on the virulence status of the APEC strain, immune status of poultry and other predisposing factors. Acute death occurs due to initial septicaemia and inflammation of multiple organs. Perihepatitis, airsacculitis, pericarditis, egg peritonitis, omphalitis etc are the most common lesions associated with colibacillosis (*The Merck Veterinary Manual*, n.d.).

There are several serotypes of APEC among them O1, O2, O5, O6, O7, O8, O11, O19, O25, O35, O73, O78, O150 and O153 are of common in poultry (Maluta et

al., 2014). As APEC has multiple serotypes, autologous bacterins may provide very limited protection against all. Identification of APEC specific virulence genes that are responsible for colonization in the intestine is out most important. APEC colonization factor *fimH* (Arné P, Marc D, Brée A, Schouler C, 2000), invasive factor *ibeA* (Germon et al., 2005) iron acquisition factor *iuc* & *iut* (Germon et al., 2005) serum resistance factor *iss* & *traT* (Pfaff-McDonough SJ, Horne SM, 2000) and antiphagocytic activities by O and K antigens present on outer membrane are the major virulence factors of APEC. O and K antigens are responsible for prevention of opsonization and phagocytosis (Li et al., 2005). Among these membrane proteins could be the possible candidate for vaccine development.

2 APEC prevention and control strategies

Prevention and control of colibacillosis on poultry relies on various aspect of management to treatment and vaccinations, however, many of the efforts are failing due to one or other factors which will be discussed below.

2.1 Biosecurity and sanitation

Proper farm and water sanitation and maintenance of biosecurity is important to reduce the entry and spread of pathogens into the farm and hatcheries. Use of appropriate disinfectants such as hypochlorites, iodophors, quaternary ammonium, formaldehyde gas, alkali and chlorhexidine from reliable companies is crucial for maintenance of farm security. Following the strict biosecurity procedures is more important to prevent the entry of pathogens into the facilities.

2.2 Breeder and hatchery management

Sanitation of parent shed, feed and water is important to prevent horizontal as well as vertical transmission of APEC (Nilsson O, Börjesson S, Landén A, 2014). Hygienic handling of hatching eggs and hatchery hygiene is equally important to prevent APEC infection in chicks.

2.3 Treatment

Judicious use of antibiotics based on antibiotic susceptibility test is the commonly used line of treatment for avian colibacillosis. Several studies showed that multi-drug resistant of APEC is of great challenge and search of new therapeutics options or prevention using effective vaccine is of urgent need.

2.4 Vaccination

Vaccination against APEC is tricky due to its several serotypes and diverse genetic background, however, multiple approaches to develop mono or polyvalent vaccine development has been initiated and some of them are commercialized. In this article, these issues will be critically analyzed and described.

2.4.1 Live-attenuated vaccines

Live vaccines are generated either deleting certain genes or inactivation by formalin. Poulvac® is live *aroA* gene deleted *E coli* serotype O78 strain EC34195 (La Ragione et al., 2013), (Sadeyen et al., 2015) which is suitable for coarse spray at day one or drinking water at day 5. Live vaccines induces both Th1 and Th2 response thereby generating strong protection (Sadeyen et al., 2015). Live vaccines provoke good immune response in oral administration and confer long lasting immunity (Singh, 2016). With the live vaccines, there are potential risk of contamination and switching to pathogenic strain.

2.4.2 Inactivated vaccines

Bacterial ghost

Bacterial ghost (BG) are the empty cell envelopes of Gram negative bacteria generated by utilizing E-lysis gene of bacteriophage PhiX147 in combination with staphylococcal nuclease A (Mayr et al., 2005). Cytoplasmic content of bacteria will be discharge via the pores generated by bacteriophage E-lysis protein leaving the membrane intact (Hajam et al., 2017). Staphylococcal nuclease A is used to degrade DNA (Mayr et al., 2005). So lack of genetic material in the bacterial ghost prevents from any adverse effect of horizontal gene transfer of resistant genes or pathogenic islands to normal microflora of gut. This membrane bound bacterial host can be used as antigen (multiple antigens). For successful utilization of BG platform, following steps should be adopted.

- ✿ **Selection of bacterial strain:** pure colony of APEC should be confirmed by cultural characteristics, biochemical tests and molecular analysis. This will help to identify the right strain which will be used to make BG.
- ✿ **Cloning of E-lysis gene:** E-lysis gene of bacteriophage PhiX147 and staphylococcal nuclease A should be amplified by conventional PCR. The full length insert genes should be cloned into the suitable vector with inducible promoter and selection markers. Plasmid vector together with E-lysis and staphylococcal nuclease A genes is called recombinant vector.
- ✿ **Transformation:** recombinant plasmid generated in previous step will be transferred to the pure colony of APEC. Overnight broth culture of APEC will be re-suspended in designated volume of ice-cold CaCl_2 to make them chemo-competent and transfer recombinant plasmid by heat-shock method at 42°C (Rahimzadeh et al., 2016).
- ✿ **Screening of transformant:** after transformation, recombinant bacterial need to identify and pick them up. For initial screening, transformants should be cultured in a medium containing respective antibiotic whose resistance gene is present in the vector. This will select both recombinant bacteria and bacteria which obtain naked plasmid without insert. To identify the recombinant bacteria we need to perform PCR against both E-lysis and staphylococcal nuclease A genes.
- ✿ **Sequencing E-lysis gene:** during cloning, inserted genes are amplified in vitro. None of the enzyme

has 100% fidelity. That's why cloned genes should be sequenced and compared with the database if there is any critical mutation during in vitro handling which may make it non-functional.

❁ **Bacterial ghost generation:** as inserted genes are under the inducible promoter, its expression is well controlled. During initial bacterial growth, there won't be expression of any E-lysis staphylococcal nuclease A. Once we achieve required number of bacteria and bacterial growth is still in log phase, we need to induce the expression of E-lysis and staphylococcal nuclease A genes (Mayr et al., 2005). E-lysis will make tunnels in the bacterial cell wall which will allow the leakage of bacterial cytoplasmic content, Staphylococcal nuclease A will degrade the bacterial DNA. Thus making bacterial ghost without nucleic acid and cytoplasmic content.

❁ **Washing bacterial ghost:** After 5-6 hrs of lysis induction, the ghost should be collected by centrifugation. Thus obtained ghost should be washed 3-4 times with 0.85% NaCl solution (Langemann et al., 2010). The washed ghosts should be suspended in distilled water and proceed for freeze-dry.

❁ **Lyophilization:** Protein E may not be 100% efficient to kill all bacteria but combination of protein E and Staphylococcal nuclease A will be effective in this process. BGs should be analyzed if there are any living-cell counts. For this lyophilized ghost preparations should be inoculated in LB and incubate for 6-7 days and analyze for living cell count by plating on LB agar.

2.4.3 Subunit vaccines

Only specific antigen from pathogen is used to elicit immune response. These vaccine candidates do not contain live components of organisms and are considered the safest type of vaccines. These vaccines evoke very strong immune response. These vaccines need booster shots to get extended protection. These vaccines are suitable for single or few serotypes, cross protection need to be verified with series of studies. To provoke effective immune response cocktail of multiple recombinant antigens can be utilized (Goor et al., 2017). To get subunit vaccine against APEC, we need to follow the following strategies.

- ❁ Identification and characterization of APEC surface protein coding gene(s)
- ❁ Amplification of target gene by PCR

- ❁ Sequencing and sequence analysis of amplicon (genomics)
- ❁ Integration into cloning vector with His-tag and inducible promoter
- ❁ Transformation and screening of transformant
- ❁ Plasmid isolation, and sequencing target genes once again if there are any crucial mutation during in vitro amplifications
- ❁ Over expression of recombinant protein (bacteria can withstand up to 60% recombinant protein)
- ❁ Purification of recombinant protein by Ni-affinity chromatography
- ❁ Characterization of purified recombinant protein
- ❁ Animal experiments (Immunological studies)

2.4.4 Membrane vesicle

Outer membrane vesicles (OMV) the small exosomes (vesicles) released by Gram-negative bacteria which contains proteolipid, cell wall components, secreted proteins (Russo et al., 2018),(Kaparakis-Liaskos & Ferrero, 2015),(Kuehn & Kesty, 2005). These vesicles show immunological activities (Tan et al., 2018). Bacteria used to release OMVs continuously but very small amount and contains endotoxins as well. Removal of endotoxin is another challenging task and will be performed by differential centrifugation (Berlanda Scorza et al., 2012). To get OMVs from APEC, we need to follow the following strategies.

- ❁ Density gradient ultra-centrifugation of broth cultured supernatant of APEC
- ❁ Characterization of vesicles: Electron microscopy
- ❁ Proteomic analysis (Western Blot) major candidate membrane proteins

2.4.5 Nucleic acid vaccines

Short synthetic ssDNA molecules containing cytosine and guanine act as immunostimulants (Weiner et al., 1997) which are abundance in microbial genomes and are the pathogen-associated molecular patterns (PAMPs) (Bauer & Wagner, 2002). PAMPs are recognize by Toll-like receptor 9 which are expressed only in B cells and dendritic cells (Rothenfusser et al., 2002). Similarly, Poly I:C is another candidate which is used for immunostimulation. It's a synthetic analog of dsDNA which interacts with toll like receptor 3 predominantly expressed in B-cells, macrophages and dendritic cells (Frank-Bertoncelj et al., 2018).

Conclusion

In nut shell, there is possibility of live APEC vaccine production by deleting pathogenic genes. Bacterial ghost platform system is another promising target for

generation of effective vaccine. Utilization of APEC outer membrane vesicles as adjuvants for effective delivery platform is possible but less commonly used target. Recent days, single or cocktail of multiple recombinant proteins are of more interest as they are more specific and less side effects.

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