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Biofilm Formation and Drinking Water Quality in Relation to *Escherichia coli* at Commercial Poultry Farms

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ABSTRACT

Biofilms are dense aggregates of surface adherent micro-organisms embedded in a poly saccharide matrix. The most alarming is the presence and multiplication of opportunistic pathogens such as *Eschericlia coli* found in biofilms. The present study was designed to determine the presence of *E. coli* in biofilm and water samples at various commercial poultry farms and to analyse factors involved in the formation of biofilm. A total of 120 biofilm and water samples were collected from inside surface of water supply network from commercial broiler and layer poultry farms in and around Faisalabad and Jhang. History of the flocks was recorded on a pre-designed questionnaire. *E. coli* was isolated form biofilm and water samples. Out of 60 biofilms samples 58 (96.6%) and out of 60 water samples 50 (83.3%) samples was found positive for *E. coli*. The quantity of bacteria in the samples was determined by acridine orange direct count and viable count. Maximum acridine orange count in biofilm and water samples was recorded 2.86×0^{9} and 1099×10^{7} mL^{-1}, respectively. Maximum viable count was 1.61×10^{9} and 1.05×10^{3} mL^{-1} in biofilm and water samples, respectively. Biofilm contributes many species of bacteria but major one is *E. coli.*

Key Words: *Escherichia coli*; Biofilm; Water quality; Chlorination; Bacterial count

INTRODUCTION

A supply of good quality water is essential for health and performance of the chicken since it constitutes 60 to 80% of live body weight (Anjum *et al.*, 1989). Biofilm is a serious threat to decrease the production as well as increase the onset of diseases. Biofilm is defined as dense aggregates of surface-adherent microorganisms embedded in a polysaccharide matrix (Siller *et al.*, 1972). Biofilm development is a result of successful attachment and subsequent growth of microorganisms on a surface.

Biofilm harbours colonies of pathogenic bacteria. Biofilm formation is a multi step process whereby bacteria, adhere to equipment surfaces, surround themselves with a protective layer of polysaccharides and grow into a network of microcolonies and water channels. The rate of biofilm formation and its release into a distribution system can be affected by many factors including surface characteristics, availability of nutrients and flow velocities. Biofilms appear to grow until the surface layers begin to slough off into the water (Geldreich & Rice, 1987).

The factors influencing biofilm formation include microbial nutrients (e.g., the concentration of carbon, nitrogen & phosphorus) in water, temperature, pipe materials, disinfectants, bacteria in water and the hydraulic regime in drinking water supply systems (Block *et al.*, 1995).

Bacteria can be introduced into water from internal and external sources. The situation where the increase of bacteria is due to internal growth or after growth of bacteria is associated with the formation of biofilms. Several investigators have shown that the multiplication of micro-organisms in biofilms along the distribution systems results in the deterioration of the bacteriological quality of drinking water, the development of odour or colour as well as the acceleration of the phenomenon of corrosion within the pipe network (Nagy & Olson, 1985).

The most alarming results of biofilm formation are the presence and multiplication of pathogenic and opportunistic pathogens such as *Escherichia coli*, *Pseudomonas*, *Mycobacteria*, *Campylobacter*, *Klebesiella*, *Aeromonas*, *Legionella* spp., *Helicobacter pylori* and *Salmonella typhimurium* occurring within the biofilms (Engle *et al.*, 1980; Wadowsky *et al.*, 1982; Burke *et al.*, 1984; Mackey *et al.*, 1998).

Therefore, the present study was designed to determine the presence of *E. coli* in biofilm and water samples at various commercial poultry farms and to analyse factors involved in the formation of biofilm.

MATERIALS AND METHODS

Collection of samples. A total of 120 (60 biofilm, 60 water) samples were collected from inside surface of water supply

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networks at various poultry farms located in the areas of Faisalabad and Jhang Districts. Two pieces (10 cm length) of water supply pipes were taken from each farm. Biofilms were removed form pipe with sterilized curator and by shaking with sterile 2 mm glass beads and rinsing twice with 5 mL sterile water. The samples were collected in sterilized test tubes containing nutrient broth and transported to laboratory under refrigeration conditions (Lehtola *et al.,* 2004).

Isolation and identification of *E. coli* from biofilm and water samples. *E. coli* was isolated by inoculating the samples on MacConkey agar which is selective medium for *E. coli* and petriplates were incubated at 37^{o}C for 24 h. Identification was done on the basis of cultural (size, shape, colour & texture of colonies), morphological and bio-chemical characteristics (Cruickshank, 1975).

Counting of Bacteria

(a). Acridine orange direct count. Biofilm and water samples were analyzed for total number of bacteria by acridine orange direct count using fluorescent microscope. Samples were stained with 0.01% acridine solution and filtered through nucleopore membrane filters. Damp filter was examined under 40 x, 100 x of fluorescent microscope (Lehtola *et al.,* 2002).

(b). Determination of viable counts for *E. coli.* The viable count was performed by spread plate method by making serial ten-fold dilutions of samples. One ml from each dilution was inoculated on separate MacConkey agar plate, incubated and count was done in plates having 30-300 colonies (Awan & Rehman, 2005).

RESULTS

Out of 120 samples, 108 samples were positive for *E. coli* collected from different Poultry Farms (Table I). *E. coli* growth was observed on MacConkey's agar. Colonies were smooth glossy, translucent and rose pink in colour. In nutrient broth, *E. coli* produced uniform turbidity. The isolates observed under microscope were Gram negative and rod shape.

After cultural and morphological characteristics, *E. coli* isolates were confirmed by biochemical reactions. Out of 120 samples, 108 samples were found positive for indole production test, methyl red test while Voges-Proskauer, citrate utilization test, urease test were found negative. *E. coli* produced gas from lactose fermentation reaction. Out of 60 biofilm samples, 58 were found positive for indole production and methyl red test. Out of 60 water samples 50 were found positive for indole production and methyl red test while Voges-prausker, Citrate utilization tests were found negative in both biofilm and water samples. Sugar fermentation reaction of *E. coli* was found positive with red colour. *E. coli* produced gas from glucose fermentation.

Table I. Isolation of *Escherichia coli* from biofilm and water samples

	Total Sample	Positive Sample
Biofilm Sample	60	58
Water Sample	60	50
Total	120	108

Acridine orange direct count. Acridine orange direct count of all samples is given in Table II. Maximum acridine orange direct count in biofilm samples was 2.86×10^9 and minimum was 2.34×10^3. Maximum count in water sample was 1.99×10^7 and minimum was 1.04×10^2.

Viable count of *E. coli.* Viable count of all samples is given in Table II. Maximum viable count of *E. coli* in biofilm samples was 1.61×10^9 and minimum was 1.05×10^3. Maximum viable count of *E. coli* in water samples was 1.03 $\times 10^7$ and minimum 0.82×10^2.

DISCUSSION

The present study indicated that 96% poultry farms have biofilm problem. *E. coli* count of these samples have shown that biofilm is one of major cause that produce colibacillosis at these poultry farms. Regular washing of pipe system and waterers with soaps and other disinfectants reduced the acridine orange direct count and viable count of *E. coli.*

At the farms where washing is done 3-4 times a day, *E. coli* count is reduced at a level that it can't produce disease. Sixty water samples were collected and 50 samples were positive for *E. coli* but acridine orange direct count and viable are less than biofilm samples. At the forms where disinfectants are used regularly formation of biofilm is less that reduced the *E. coli* count in water and biofilm samples. This study is in agreement with the findings of Lehotla *et al.* (2004) that use of chlorine regularly reduced the formation of biofilm in water distribution networks. Those water samples in which disinfectants are added are negative for *E. coli* and if *E. coli* were present then count was less than biofilm.

According to Pakistan Research Council on Water Quality and British Ministry of Health (1970) the threshold level of *E. coli* in drinking water is 10 bacteria per 100 mL of water. In present study the maximum acridine orange direct count in biofilm is 2.86×10^9 per ml and minimum was 2.34×10^3 mL^{-1}. Maximum count in water sample is 1.99×10^7 and minimum 1.04×10^2. These counts are so high than the threshold level. Maximum viable count of biofilm are 1.03 X 10^7 and minimum was 0.82 10^2 mL^{-1}.

A few numbers of bacteria in water may lead to biofilm formation early as compared to completely pure sterile water. Chlorination reduces the biofilm formation. Biofilm count increases by the entry of bacteria in pipe system through breakage of pipeline that increases biofilm problem.

It was also observed that the development of odour or colour as well as the acceleration of the phenomenon of corrosion within pipe may result in increase of *E. coli* counts in biofilm and water samples. Nagy and Olson (1985) have also observed the same factors for the prevalence of *E. coli* in biofilm samples.

Table II. Acridine Orange Direct Count and viable count of different samples in Jhang

Sample #	AODC		Viable count		Location
	B	**W**	**B**	**W**	
1	2.17×10^8	1.56×10^4	2.17×10^8	1.56×10^4	Chenab College Jhang
2	2.08×10^6	1.47×10^3	2.08×10^6	1.47×10^3	Chak # 174
3	1.99×10^6	1.47×10^3	1.99×10^6	1.47×10^3	Chak # 175
4	2.52×10^6	1.47×10^2	2.52×10^6	1.47×10^2	Chak # 266
5	Negative	Negative	Negative	Negative	Aminpur Road
6	2.25×10^6	Negative	2.25×10^6	Negative	Jhang Sadar
7	1.73×10^6	1.30×10^5	1.73×10^6	1.30×10^5	Jhang Sadar
8	2.86×10^6	Negative	2.86×10^6	Negative	Chak # 214
9	2.60×10^8	1.56×10^7	2.60×10^8	1.56×10^7	Chak # 215
10	2.69×10^8	1.56×10^6	2.69×10^8	1.56×10^6	Chak # 172
11	2.78×10^8	1.99×10^6	2.78×10^8	1.99×10^6	Chiniot mor
12	2.08×10^8	1.99×10^6	2.08×10^8	1.99×10^6	Mochiwala
13	1.99×10^5	1.12×10^3	1.99×10^5	1.12×10^3	Toba Road Jhang
14	2.43×10^9	1.39×10^7	2.43×10^9	1.39×10^7	Satellite Town
15	2.86×10^6	1.99×10^7	2.86×10^6	1.99×10^7	Hassan Nagar

AODC, Acridine Orange Direct Count; B, Biofilm; W, Water

Table III. Acridine Orange Direct Count and viable count of different samples in Ahmad pur Sial

Sample #	AODC		Viable count		Location
	B	**W**	**B**	**W**	
1	2.08×10^9	1.39×10^6	2.08×10^9	1.39×10^6	Ahmad Pur Sial
2	2.25×10^9	1.39×10^6	2.25×10^9	1.39×10^6	Garh Mor
3	Negative	Negative	negative	Negative	Garh Maharaja
4	2.17×10^4	1.30×10^4	2.17×10^4	1.30×10^4	Hassu Balail
5	2.34×10^3	1.30×10^2	2.34×10^3	1.30×10^2	Meernewala
6	2.34×10^3	1.30×10^2	2.34×10^3	1.30×10^2	Sial Chowk
7	2.17×10^4	1.30×10^2	2.17×10^4	1.30×10^2	Gudara
8	2.25×10^5	1.39×10^2	2.25×10^5	1.39×10^2	Dowloana
9	2.43×10^6	1.39×10^4	2.43×10^6	1.39×10^4	Durigondal
10	2.34×10^7	1.47×10^5	2.34×10^7	1.47×10^5	Samanduana
11	2.34×10^7	Negative	2.34×10^7	Negative	Bangla Yasmin
12	2.34×10^7	1.56×10^6	2.34×10^7	1.56×10^6	Rodu Sultan
13	2.34×10^7	1.56×10^6	2.34×10^7	1.56×10^6	Haeli Jaiwain
14	2.25×10^7	Negative	2.25×10^7	Negative	Mehmood Kot
15	2.17×10^6	2.17×10^4	2.17×10^6	2.17×10^4	18 Hazari
16	2.34×10^6	2.17×10^6	2.34×10^6	2.17×10^6	Sharifabad
17	2.34×10^8	1.99×10^4	2.34×10^8	1.99×10^4	Kot Mupal
18	2.43×10^6	1.99×10^5	2.43×10^6	1.99×10^5	Kot Umar Daraz
19	2.17×10^6	1.30×10^4	2.17×10^6	1.30×10^4	Chak # 268
20	2.17×10^6	1.39×10^4	2.17×10^6	1.39×10^4	Ranjit Kot
21	2.17×10^6	Negative	2.17×10^6	Negative	Garh Mahaaja City

AODC Acridine Orange Direct Count; B, Biofilm; W, Water

Table IV. Acridine Orange Direct Count and viable count of different samples in Faisalabad

Sample #	AODC		Viable count		Location
	B	**W**	**B**	**W**	
1	2.17×10^6	1.39×10^6	2.17×10^6	1.39×10^6	Sheikhupura Road
2	1.99×10^7	1.00×10^4	1.99×10^7	1.00×10^4	Chak # 102 RB
3	2.25×10^7	1.30×10^4	2.25×10^7	1.30×10^4	Chak # 103 RB
4	2.34×10^7	1.56×10^4	2.34×10^7	1.56×10^4	UAF
5	2.78×10^6	1.99×10^5	2.78×10^6	1.99×10^5	Sammundri Road
6	2.25×10^6	Negative	2.25×10^6	Negative	Chak # 289 RB
7	2.25×10^6	Negative	2.25×10^6	Negative	Chak # 247 RB
8	2.17×10^5	1.56×10^4	2.17×10^5	1.56×10^4	Chak # 232 RB
9	2.43×10^7	1.12×10^3	2.43×10^7	1.12×10^3	Roshan Wala
10	1.56×10^7	1.12×10^4	1.56×10^7	1.12×10^4	Jhang Bypass
11	1.65×10^7	1.04×10^4	1.65×10^7	1.04×10^4	Chak # 233 RB
12	1.65×10^7	1.04×10^3	1.65×10^7	1.04×10^3	Chak # 242 RB
13	2.17×10^6	1.04×10^3	2.17×10^6	1.04×10^3	Chak # 244
14	1.99×10^6	1.12×10^3	1.99×10^6	1.12×10^3	Ghulam M. Abad
15	2.43×10^6	1.47×10^3	2.43×10^6	1.47×10^3	Chak # 279 RB
16	2.25×10^6	1.30×10^3	2.25×10^6	1.30×10^3	Chak Jhumra

AODC, Acridine Orange Direct Count; B, Biofilm; W, Water

Table V. Acridine Orange Direct Count and viable count of different samples in Chiniot

Sample #	AODC		Viable count		Location
	B	W	B	W	
1	2.69×10^7	1.39×10^4	2.69×10^7	1.39 × 104	Chiniot City
2	2.69×10^7	1.04×10^5	2.69×10^7	1.04 × 105	Langrana
3	2.60×10^7	Negative	2.60×10^7	Negative	Chak Jappa
4	2.78×10^5	1.04×10^2	2.78×10^5	1.04×10^2	Bhoana
5	2.25×10^5	1.12×10^2	2.25×10^5	1.12×10^2	Mozah Chadhar
6	2.08×10^5	1.12×10^2	2.08×10^5	1.12×10^2	Mozah Mangeeni

AODC, Acridine Orange Direct Count; B, Biofilm; W, Water

Table VI. Acridine Orange Direct Count and viable count of different samples in Shorkot

Sample #	AODC		Viable count		Location
	B	W	B	W	
1	1.99×10^5	1.30×10^4	1.99×10^5	1.30×10^4	Shorkot City
2	2.86×10^5	1.47×10^4	2.86×10^5	1.47×10^4	Shorkot City

AODC, Acridine Orange Direct Count, B, Biofilm; W, Water

Biofilm is a serious threat to poultry industry in Pakistan and majority of poultry farms have biofilm problem due to lack of management. Biofilm contributes many species of bacteria but major one is *E. coli.* Basic and main source of *E. coli* infection at commercial broiler and layer poultry farms is biofilm formation and contaminated water and this problem may be solved by the regular use of water disinfectants and removal of biofilm from water distribution networks.

CONCLUSION

Biofilm is a serious threat to poultry industry in Pakistan and majority of poultry farms have biofilm problem due to lack of management. Biofilm contributes many species of bacteria but major one is *E. coli.* Basic and main source of *E. coli* infection at commercial broiler and layer poultry farms is biofilm formation and contaminated water and this problem may be solved by the regular use of water disinfectants and removal of biofilm from water distribution networks.

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