Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.066

SHORT COMMUNICATION

Rapid Detection of Biofilm Formation by Zoonotic Serovars of *Salmonella enterica* and Avian Pathogenic *E. coli* Isolates from Poultry

Shiza Nawaz[1,2], Muhammad Moman Khan[2], Jonas Noack[2], Asad Bashir Awan[1], Juliane Schiebel[2], Dirk Roggenbuck[2,3], Peter Schierack[2], Yasra Sarwar[1] and Aamir Ali[1]*

[1]National Institute for Biotechnology and Genetic Engineering (NIBGE), Constituent College of Pakistan Institute of Engineering and Applied Sciences (PIEAS), Faisalabad, Pakistan, [2]Institute for Biotechnology, Brandenburg University of Technology (BTU) Cottbus-Senftenberg, Senftenberg, Germany, [3]Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology Cottbus – Senftenberg, the Brandenburg Medical School Theodor Fontane and the University of Potsdam, Germany
*Corresponding author: myaamirali@yahoo.com

ARTICLE HISTORY (20-301)

Received: June 13, 2020
Revised: July 13, 2020
Accepted: July 13, 2020
Published online: July 25, 2020

Key words:
APEC
Biofilm formation
Salmonella
VideoScan technology

ABSTRACT

Biofilms are complex, sessile microbial communities that are problematic in clinical settings due to their association with survival and pathogenicity of bacteria. The biofilm formation supporting conditions for zoonotic serovars of *Salmonella* and avian pathogenic *E. coli* (APEC) from poultry have not been well studied yet. Clinical isolates of zoonotic *Salmonella* and APEC from poultry were evaluated for biofilm formation in four media at 37°C and 40°C after incubation of 48 and 72 hrs. The biofilms formed in 96 well plates were visualized and quantified with a new module of Aklides system using fluorescence microscope coupled with automated VideoScan Technology. After 72 hrs, brain heart infusion at 40°C and Rappaport-Vassiliadis Soya broth at 37°C were found most suitable for APEC and *Salmonella* biofilm formations respectively. The new information will be useful for further biofilm associated studies particularly for evaluation of antibiofilm compounds and contribute in infection control.

To Cite This Article: Nawaz S, Khan MM, Noack J, Awan AB, Schiebel J, Roggenbuck D, Schierack P, Sarwar Y and Ali A, 2020. Rapid detection of biofilm formation by zoonotic serovars of *Salmonella enterica* and avian pathogenic *E. coli* isolates from poultry. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2020.066

INTRODUCTION

Biofilms are defined as a population of bacterial cells producing an extracellular polysaccharide matrix (EPM) that helps in their persistence against harsh environmental conditions. Biofilms formed by multiple bacterial species, particularly on medical devices, cause different infections and pose a great threat to health. Poultry is among major industries of Pakistan and the resistance against the antimicrobials used at poultry farms is due to multiple factors including biofilm formation by the prevailing bacteria. Among the zoonotic bacteria of poultry, avian pathogenic *E. coli* (APEC) and *Salmonella* spp. are very important. APEC causes substantial economic losses because it is responsible for different respiratory and other systemic diseases at poultry farms (Kabir, 2010). Different zoonotic serovars of *Salmonella enterica* (*S.* Typhimurium, *S.* Enteritidis and *S.* Infantis) cause infections in poultry as well as in humans through food products. *Salmonella* infections in poultry cause great economic losses by decrease in egg production, reduced growth and even mortality (Dar *et al.*, 2017). The biofilm-associated bacteria can be more resilient to antibiotics and host immune system (Vestby *et al.*, 2020), and also the extracellular substances produced by *Salmonella* biofilms are hypothesized to prevent pathogen clearance (Hahn and Gunn, 2020). There is variability among different serovars of *Salmonella* and APEC regarding biofilm formation on different surfaces. There is limited information regarding biofilm formation potential of different zoonotic serovars of *Salmonella* and APEC in different growth conditions.

MATERIALS AND METHODS

Clinical isolates from poultry: *S.* Typhimurium (n=10), *S.* Infantis (n=10), *S.* Enteritidis (n=4), (originally isolated from Faisalabad, Pakistan) and APEC (n=16) were taken from glycerol stock cultures of the Brandenburg University of Technology Cottbus-Senftenberg (BTU, C-S), Germany. These isolates were

revived in Luria-Bertani (LB) broth by incubating overnight at 37°C. The bacterial identification was performed using CHROMagar™ Orientation (MASTGRP, Germany) plates and the confirmation was done by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis (microflex LT/SH; Bruker Daltonic, Germany) following standard procedures (Wajid *et al.*, 2019). The biofilm formation assay of these isolates was performed in four different growth media including Rappaport-Vassiliadis Soya (RVS) Peptone broth (Oxoid, UK, CM0866), tryptic soy broth (TSB) (Oxoid, UK, CM0129), Brain Heart Infusion (BHI) broth (Oxoid, UK, Cat# CM1135) and M63 broth (minimal medium). The overnight bacterial growth of each isolate was diluted 1:100 in respective media (RVS, TSB, BHI and M63) and 100 µL of the suspension was dispensed in respective wells of the 96-well polystyrene microtiter plate (Greiner, Germany, Cat # 655161). The assay was performed at two different incubation temperatures viz. 37°C and 40°C for two different incubation times viz. 48 hrs and 72 hrs. Each isolate was tested in triplicate wells for each media and the assay was performed twice. *E. coli* strain K-12 MG1655 F'tet *traD* served as positive control while the negative control wells only contained sterile media. After incubation, the 96 well plates were aspirated and each well was washed with 100 µL of 0.9% NaCl to remove the non-adherent bacteria. A 50 µL volume of SYTO9 (5µM) green fluorescent nucleic acid stain (Thermo Scientific, Cat# S34854) was added in each well and incubated in dark for 10 min. A washing step was performed with 100 µL of 0.9% NaCl. Finally, the 100 µL of 0.9% NaCl was added in each well except wells B_1, C_1 and D_1 (left blank for addition of fluorescent reference beads). In each reference well (B_1, C_1 and D_1), 100 µL of coumarin/rhodamine-coded reference microbeads (PolyAn GmbH, Berlin, Germany) suspended in 0.9% NaCl were added. Each plate was analyzed for biofilm formation using fluorescence microscope coupled with the VideoScan Technology (Schiebel *et al.*, 2017). For the VideoScan, a modified module of Aklides system was used for live imaging of the bacterial biofilms in fluorescein isothiocyanate (FITC) fluorescent channel and the FastFluoScan software was used to measure fluorescence intensities of the biofilms. The system after taking images of each well, also gave the corresponding fluorescence values of each well and an overall picture of the whole plate as described previously (Awan *et al.*, 2019, Schiebel *et al.*, 2017). The representative images made during the study are shown in Fig. 1. After data analysis based on the Stepanovic method (three standard deviation descriptive statistics), the bacterial isolates were classified into four respective groups: non-biofilm producers, weak biofilm producers, moderate biofilm producers and strong biofilm producers (Stepanović *et al.*, 2000).

RESULTS AND DISCUSSION

All the APEC isolates, when grown in BHI media showed strong or moderate biofilm formation at both temperatures and the quality of biofilm formation improved with the increase in incubation time. However, in TSB and M63 broths, the APEC isolates formed a varying degree of biofilms at 40°C after 72 hrs of incubation but not in RVS broth. The *S.* Typhimurium and *S.* Infantis isolates showed strong biofilm production at 37°C in all the four media particularly after 72 hrs and the maximum biofilm formation was observed in RVS media at all conditions. In BHI medium, strong biofilm was formed by *S.* Infantis at 40°C after 72 hrs while in M63 medium, strong biofilm formation was observed at 37°C after 48 hrs of incubation. All *S.* Enteritidis isolates formed strong or moderate biofilms in all enriched and minimal media at 37°C after 72 hrs except one isolate which showed weak biofilm formation in RVS medium. The biofilms formed by the APEC and *Salmonella* isolates used in the study are shown in Fig. 2.

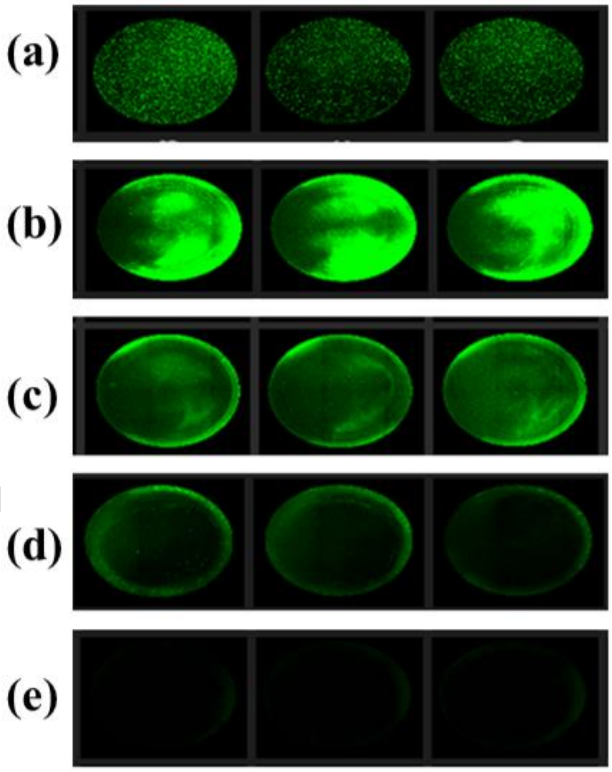

Fig. 1: Biofilm detection by VedioScan Technology: The microscopic images of (a) Flourescence reference beads, (b) Strong biofilm, (c) Moderate biofilm, (d) Weak biofilm and (e) No biofilm.

There is a strong prevailing presumption that the formation of microbial communities is associated with their pathogenicity and the bacteria residing in biofilms are main cause of persistent and chronic infections. Furthermore, the antibiotics currently available are ineffective to target biofilm related infections due to their higher levels of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) approaching to the minimum toxic concentrations (MTC). Hence, it is critically important to design and screen new anti-biofilm molecules that can work independently or synergistically with other antimicrobials. We have recently developed the VideoScan Technology and optimized it for fast and high throughput screening of biofilms formed by *E. coli* (Schiebel *et al.*, 2017), *Pseudomonas* (Awan *et al.*, 2019) isolates and now for recently isolated (Wajid *et al.*, 2019) zoonotic serovars of *Salmonella.*

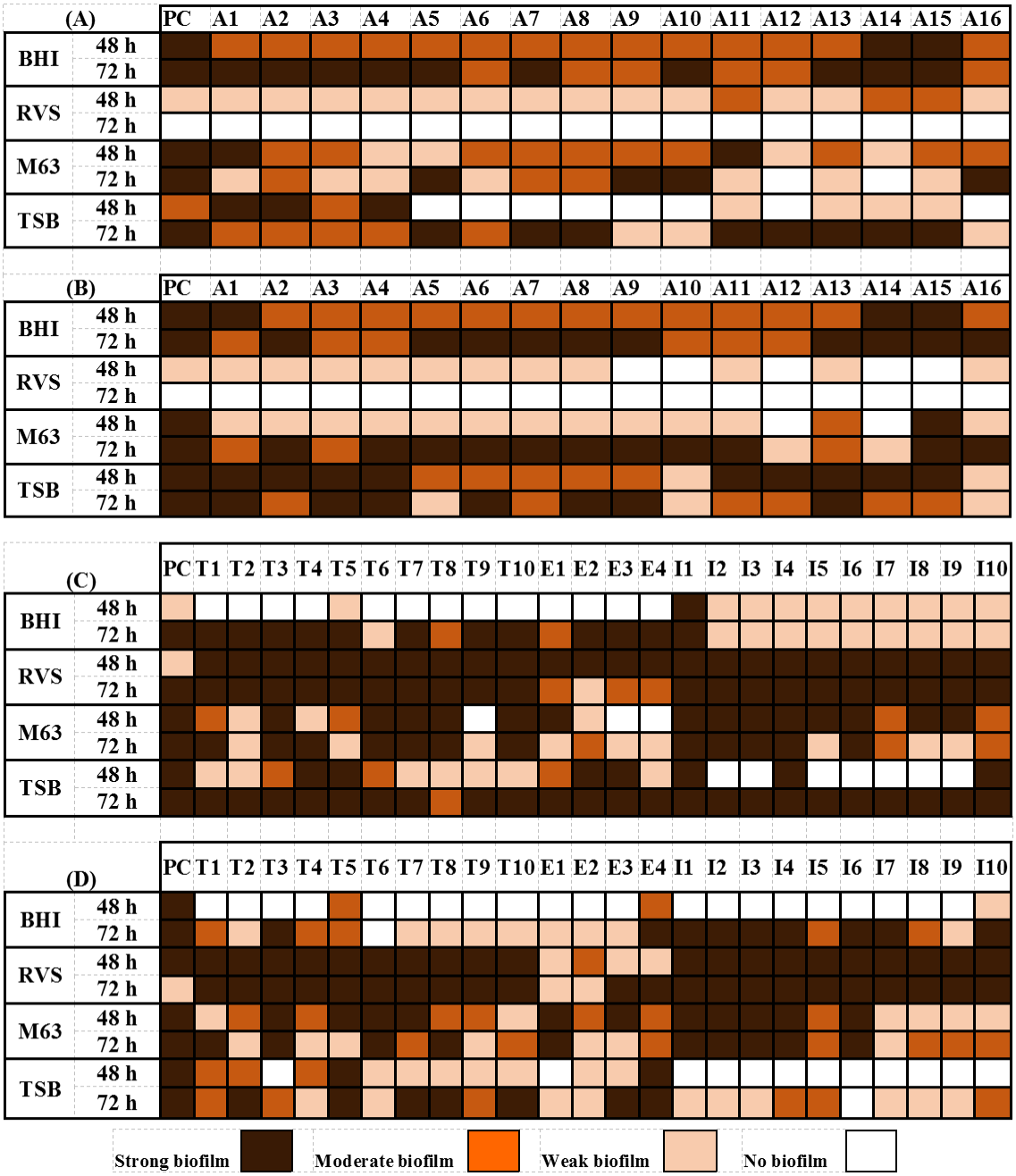

Fig. 2: Biofilm formation by APEC and *Salmonella* under different conditions: (A) APEC at 37°C, B) APEC at 40°C, (C) *Salmonella* at 37°C and (D) *Salmonella* at 40°C. The letters A is abbreviated for APEC, T for *S.* Typhimurium, E for *S.* Enteritidis, I for *S.* Infantis and PC for positive control.

The observed strong biofilm formation by *Salmonella* in RVS media is in line with the previous report (Chandra *et al.*, 2017) where RVS has been found to support *Salmonella* growth. It is therefore understandable that no strong biofilm was produced by APEC isolates in RVS media. We observed weak or no biofilm production by *Salmonella* in BHI media (Fig. 2). However, we found BHI (enriched) and M63 (minimal) media at 40°C for 72 hrs incubation as most suitable conditions for biofilm formation by the APEC isolates. The BHI and M63 media has been previously reported as suitable for biofilm production by the APEC isolates (Skyberg *et al.*, 2007). This study revealed the high biofilm formation potential among APEC isolates and moderate biofilm formation potential among *Salmonella* isolates suggesting APEC as better survivor in the environment.

Overall, the automated Aklides system using fluorescence microscope coupled with VideoScan imaging technology was found as an excellent tool for fast and high throughput screening of biofilm formation by *Salmonella* and APEC isolates. This tool can further be utilized to screen a number of bacteria to find the optimum biofilm forming conditions including different media, incubation temperatures, surface materials and time durations. The particular advantage of this technique is visualization and imaging of the produced biofilms that make the technique preferable over crystal violet staining. A next step should be testing biofilm formation under natural conditions: drinking water, temperatures like at poultry farms, on grains of sand or on metals used for water tubes, cages etc. The new information regarding optimum conditions for biofilm formation by APEC and

Salmonella can be utilized in further biofilm-associated studies including the screening of different existing or novel anti-biofilm compounds alone and/or in combination with various antimicrobials.

Acknowledgments: This work was supported by project 'FISH für Tumor und Erregerdiagnostik mittels VideoScan (03PSZZFIA) funded by the Federal Ministry of Education and Research (BMBF, Germany).

Authors contribution: SN and MMK performed the biofilm and MALDI-TOF-MS experiments. JN, JS and DR optimized and analyzed the VideoScan modules, experiments and results. ABA and YS performed the microbiology and statistical work. PS and AA conceived, designed and supervised the overall study. All authors have contributed in writing and improving the manuscript with intellectual inputs and approved the final version.

REFERENCES

Awan AB, Schiebel J, Böhm A, *et al.,* 2019. Association of biofilm formation and cytotoxic potential with multidrug resistance in clinical isolates of *Pseudomonas aeruginosa*. EXCLI J 18:79-90.

Chandra M, Thakur S, Narang D, *et al.,* 2017. Evaluation of *Salmonella* Typhimurium biofilm against some antibiotics. Indian J Experim Biol 55:562-7.

Dar M, Ahmad S, Bhat S, *et al.* 2017. *Salmonella* Typhimurium in poultry: a review. World Poult Sci J 73:345-54.

Hahn MM and Gunn JS, 2020. Salmonella Extracellular Polymeric Substances Modulate Innate Phagocyte Activity and Enhance Tolerance of Biofilm-Associated Bacteria to Oxidative Stress. Microorganisms 8:253.

Kabir LMS. 2010. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int J Environ Res Public Health 7:89-114.

Schiebel J, Böhm A, Nitschke J, *et al.,* 2017. Genotypic and phenotypic characteristics associated with biofilm formation by human clinical *Escherichia coli* isolates of different pathotypes. Appl Environ Microbiol 83:e01660-7.

Skyberg J, Siek K, Doetkott C, *et al.,* 2007. Biofilm formation by avian *Escherichia coli* in relation to media, source and phylogeny. J Appl Microbiol 102:548-54.

Stepanović S, Vuković D, Dakić I, *et al.,* 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 40:175-9.

Vestby LK, Grønseth T, Simm R, *et al.,* 2020. Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiotics 9:59.

Wajid M, Awan AB, Saleemi MK, *et al.,* 2019. Multiple Drug Resistance and Virulence Profiling of *Salmonella enterica* Serovars Typhimurium and Enteritidis from Poultry Farms of Faisalabad, Pakistan. Microb Drug Resist 25:133-42.