Bacterial fibers critical to human, avian infection

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Biodesign researchers (from to left) Alyssa Stacy, Jacob Maddux, Natalie Mitchell and team leader Melha Mellata. Credit: The Biodesign Institute

(Medical Xpress)—Escherichia coli – a friendly and ubiquitous bacterial resident in the guts of humans and other animals – may occasionally colonize regions outside the intestines. There, it can have serious consequences for health, some of them lethal.

In a new study conducted in assistant professor Melha Mellata's lab in the Biodesign Institute at Arizona State University, lead author Alyssa K. Stacy and her colleagues examine one such bacterial adversary, Avian pathogenic Escherichia coli (APEC).

The research, conducted in collaboration with scientists at the University of Florida, Gainesville, appears in the current issue of the journal *PLOS ONE*.

The researchers targeted a specific group of threadlike fibers known as E. coli common pilus (ECP), which adorn bacterial cell surfaces. In the first study of its kind, they analyzed the way these structures contribute to APEC's ability to cause infection and form dense cell aggregates known as biofilms.

APEC infections are a serious threat to poultry, causing both systemic and localized infections, collectively known as colibacillosis. These afflictions cause significant economic losses to the poultry industry, due to the costs of treatment for infected birds, lowered rates of egg production and mortality.

Further, APEC infections may pose a risk to humans, due to their zoonotic potential – their ability to infect human hosts. A better understanding of infectious capacity (or virulence) and zoonotic potential are therefore essential for combatting these hazardous pathogens.

Stacy was an undergraduate student in Mellata's lab, and was partially supported by funding from School of Life Sciences Undergraduate Research (SOLUR), ASU. She was joined by Biodesign researchers Natalie M. Mitchell, Jacob T. Maddux and Roy Curtiss III, director of the institute's Center for Infectious Diseases and Vaccinology.

Avian Pathogenic E. coli belong to a broad group of extraintestinal pathogenic E. coli (ExPEC) strains. Colibacillosis, caused by APEC in birds, leads to serious illness, often attacking the avian respiratory system, producing systemic or localized infections depending on the age and gender of bird, immunologic health and various environmental factors.

Because APEC and human ExPEC forms share important virulence characteristics, possible zoonotic transmission is a serious health concern. APEC may also provide a reservoir for virulence genes that may be acquired by human strains.

Many types of bacteria produce extracellular surface fibers like ECP, enabling them to adhere to one another, as well as to various surfaces. But such fibers – or pili – perform other vital functions, particularly in the case of pathogenic bacteria. Pili, including those projecting from the surfaces of E. coli, are capable of recognizing specific host cell receptors during their initial phase of colonization.

Bacteria make further use of their pili to form cellular biofilms. Such bacterial aggregates are of clinical importance, as they provide reservoirs for pathogenic organisms to persist in the host, and often display increased resistance to antibiotics.

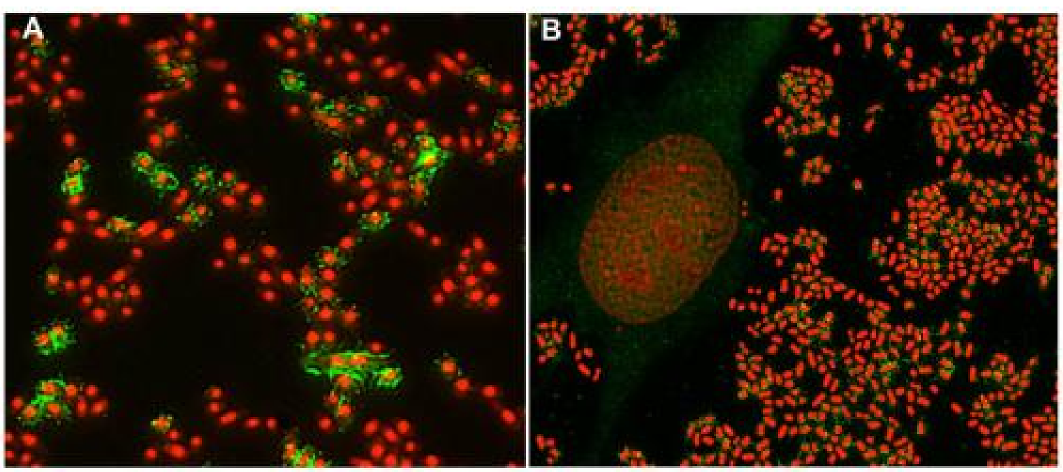

Figure 1 shows the bacterial expression of *Escherichia coli* common pilus (ECP) using fluorescence (in green) in biofilm (A) and in contact with cells (B). Both commensal and pathogenic *E. coli* strains produce ECP. In Avian pathogenic *E.*

coli (APEC) infections, ECP plays an important role in host cell recognition and biofilm formation. Credit: The Biodesign Institute at Arizona State university

E. coli common pilus was originally identified in an ExPEC form known to cause neonatal meningitis in humans, but was later recognized as a component in all classes of E. coli – both pathogenic and benign.

While E. coli bacteria exist primarily as beneficial residents of the human intestine, extraintestinal variants are responsible for diarrheal diseases like hemorrhagic colitis, as well as urinary tract infections, neonatal meningitis, sepsis and pneumonia. The toll of such diseases – particularly in the developing world – is substantial, claiming some 2.5 million lives per year. Most of these victims are children.

The current study draws on examinations of ECP, both in vitro and in vivo. The aim was to determine the prevalence of ECP among APEC strains and evaluate its contribution in the early stage of biofilm formation and host-cell recognition. Additionally, the study assessed ECP's role in virulence in baby chicks.

The new research demonstrates – for the first time – the prevalence of ecpA, a gene coding for a major structural subunit of ECP in a majority APEC sequences examined. (The complex architecture of ECP fibers is composed of six distinct structural subunits.) With the aid of PCR methods, the group tested 167 APEC strains derived from chickens and turkeys afflicted with colibacillosis, 76 percent of which tested positive for ecpA (which was previousely associated with human pathogenic E. coli).

The authors stress that the results confirm that APEC and human pathogenic E. coli strains share virulence traits. They further speculate that ecpA may permit the persistence of E. coli bacteria in the intestine, where they exist in a non-threatening state, before migrating to alternate, extraintestinal sites and becoming pathogenic.

Environmental conditions, including low pH, low growth temperature and high acetate concentration have been shown to upregulate the expression of ECP in human E. coli strains that cause urinary tract infections, meningitis and diarrheal diseases. In the current study, an APEC strain was found to adhere to human cervical cells in a manner similar to human ExPEC infections. Further, the results showed that adorning APEC with anti-ECP antibodies – a process known as opsonization – could significantly inhibit bacterial adherence. This finding suggests that ECP could be considered as a potential antigen for vaccines for both human and poultry infections.

The formation of biofilms is a common bacterial property, including in E. coli, where the adaptation increases survivability inside and outside of the host, and provides an ideal environment for the exchange of genetic material. Bacteria forming biofilms frequently display antibiotic resistance, and can be tenacious foes to combat medically. Deletion of ECP-related genes was shown to reduce biofilm production.

Finally, the study attempted to evaluate APEC virulence in baby chicks, using strains with deleted ECP genes. Results show a reduction in virulence. In fact, the potential for colonization among the ECP deletion strains was reduced, particularly in the bloodstream.

The new work demonstrates multiple roles for ECP in APEC, and thus presents a plausible target for future therapeutics aimed at these serious infections of both humans and animals.

"Our study has clearly shown that although the gene of ECP was found in a large number of APEC, these bacteria express this gene differently when they are in contact with cells or in biofilm," Mellata says. "Elucidating how the expression of some genes is turned on or off by different factors will help us understand how these bacteria cause disease."

More information: Stacy AK, Mitchell NM, Maddux JT, De la Cruz MA, Durán L, et al. (2014) "Evaluation of the Prevalence and Production of Escherichia coli Common Pilus among Avian Pathogenic E. coli and Its Role in Virulence." *PLoS ONE* 9(1): e86565. DOI: 10.1371/journal.pone.0086565

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