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Identifying virulence factor genes in *E. coli* in animal houses and their transmission to outside environments

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ABSTRACT

Escherichia coli is a common bacterium in the air; it can affect the health of humans and animals. The carrying status of five primary virulence factor genes in *E. coli* (i.e., STa, STb, LTa, stx1, and stx2/stx2e) in various animal houses and their spread to the outside environment were investigated by comparing the air inside animal houses with that outside the houses. Multiplex polymerase chain reaction detection of the five genes was performed on samples from five chicken houses, and 117 *E. coli* isolates were obtained. From five swine houses, 120 were obtained, and from six cow houses, 143 were obtained. Results reveal that *E. coli* virulence genes derived from animal houses can be propagated via gas exchange from the inside to the outside and, depending on weather conditions, across some distance, resulting in biological pollution and the spread of pathogenic microorganisms. This study of the transmission of environmental microbial aerosols in animal houses has great significance in public health and epidemiology.

1. Introduction

Although air is vital for humans and other animals to survive, in most cases, the microbes that affect human and animal health and production performance are airborne. Accordingly, measuring microbial air contamination is essential for gauging air quality. At the same time, microbes are a chief factor of environmental pollution in animal houses, where pollution can cause the spread of a number of infectious diseases. Airborne bacteria in animal houses include pathogenic, conditionally pathogenic, and nonpathogenic bacteria (Dowes, Thorne, Pearce, & Heederik, 2003) that can cause infection among both animals and animal breeders (Dutkiewicz, Pomorski, & Sitkowska, 1994; Fiegel, Clarke, & Edwards, 2006). Even a small amount of pathogenic bacteria can cause respiratory tract infections, especially in the lower respiratory tract (Donaldson, 1999; Fiegel et al., 2006). Therefore, microbial aerosol pollution not only affects human and animal health, but also results in the spread of infectious zoonotic pathogens among humans and animals (Duan, 2005; Dutkiewicz, 1994).

A commensal bacterium in the intestinal tracts of humans and animals (Schroeder, White, & Meng, 2004), *Escherichia coli* can cause colibacillosis under certain conditions, and it contaminates the environment via fecal excretion (Beutin, Steinrück, Krause, Steege, & Haby, 2007; Jackson, Blair, McDowell, Kennedy, & Bolton, 2007; Somarelli, Makarewicz, Sia, & Simon, 2007). Moreover, it

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is a common bacterium in the air, especially in animal houses and their surrounding environments. *Escherichia. coli* is therefore an important pathogenic bacteria in water, food, and other agricultural and environmental forms of pollution (Duan, Chai, Wang, Müller, & Zucker, 2006; Zucker, Trojan, & Muller, 2000).

Pathogenic *E. coli* must first be localized in the settlement and breeding and then invade the body or produce toxins. Not all virulence factors of the various pathogenic *E. coli* are the same. For example, ST and LT enterotoxins, as well as others, can change the absorption and secretion functions of intestinal cells, resulting in large water and electrolyte flows to the intestine–the clinical manifestation of watery diarrhea–or vascular endothelial cell damage, resulting in piglet diarrhea and edema disease. The most potent placental immunogen and mucosal immune adjuvant is LT, and it can effectively start the body's local and systemic humoral immunity and cellular immunity. On the other hand, Stx has strong cytotoxic, enterotoxic, and neurotoxic effects, which cause different clinical manifestations.

Aerosols composed of microbes and their metabolites in and around animal houses cause environmental pollution (Gross, 1994), influence animal health and productivity, and prompt the spread of infectious airborne diseases (Brown & Hovmoller, 2002; Lv et al., 2015). Research on the spread of microbial aerosols in animal breeding environments has focused on changes in bacterial concentrations (Hwang, Yoon, Ryu, Paik, & Cho, 2010; Kim & Kim, 2007), bacterial resistance, and the content of certain pathogenic bacteria (Cattoir, Poirel, Rotimi, Soussy, & Nordmann, 2007; Ibenyassine, AitMhand, Karamoko, Cohen, & Nennaji, 2006); however, studies conducted in the field from a molecular biology perspective are rare (Gao et al., 2015). In response, we examined the carrying situations of five primary virulence factor genes in *E. coli* inside and outside various animal houses. We also investigated the spread of the genes to the outside of the houses by performing comparative detection analyses on the genes found inside and outside the houses. The spread and infection of *E. coli* microbial aerosols present a great threat to the health of residents around poultry houses, especially those downwind. This study has strong public health and epidemiological significance.

2. Materials and methods

2.1. Animal house situations

Sampling sites were at several farms in the cities of Taian, Laiwu, Jinan, and ZiBo, China. Samples were taken from five chicken houses, five swine houses, and six cow houses, each house utilizing a different structure type. Wind speed, temperature, and relative humidity were measured at the beginning, middle, and end of the sampling period. Table 1 presents detailed information about the animal houses.

Table 1
Description of the animal houses.

Animal houses		Sampling sites	Total number	Breeding ways	Inside houses			Outside houses		
					Temperature (°C)	Humidity (%)	Wind speed (m/s)	Temperature (°C)	Humidity (%)	Wind speed (m/s)
Chicken houses	A	Chicken farm in Ningyang	6000	closed	26	40	0	21	50	1–3
	B	Fangcun, Taian 1	2200	caged	26	34	0	29	50	1–3.1
	C	Fangcun, Taian 2	3000	half-closed	31	44	0	35	36	1.5–3
	D	Mazhuang, Taian	3500	half-closed	31	60	0	32	75	0–1.5
	E	Xuezhuang, Taian	4500	caged	30	70	0	31	65	0–2
Swine houses	A'	Taian 1	150	closed	7	81	0	4	78	0–0.6
	B'	Niuquan, Laiwu	500	half-closed	12	65	0	8	60	1.4–5
	C'	Mazhuang, Feicheng	600	half-closed	28	67	0	35	58	0–1.5
	D'	Taian 2	360	half-closed	20	67	0	22	70	0–1.5
	E'	Taian 3	280	half-closed	22	65	0	24	70	0–1.5
Cow houses	A″	Cow farm in Taian 1	1000	half-closed	30	65	0	32	60	0–1.5
	B″	Cow farm in Taian 2	450	half-closed	24	65	0	26	60	1–5
	C″	Jinan	300	open	22	67	0	24	65	0–1.5
	D″	Gaoqing, Zibo 1	520	half-closed	20	63	0	21	60	0–2.5
	E‴	Gaoqing, Zibo 2	600	half-closed	19	63	0	20	62	0–1.5
	F″	Cow farm in Taian 3	500	open	16	58	0	18	52	0–2

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