ANTIBIOTIC RESISTANCE OF AIRBORNE *ESCHERICHIA COLI* FROM HEN HOUSE AND RABBITRY AND THEIR SPREADING TO SURROUNDINGS

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ABSTRACT

The Indoor and outdoor (windward 100 m and downwind 10, 50, 100, 200, 400, 600, 800 m) air samples in one rabbitry and one hen house in Shandong province, China were collected by two 6-stage Andersen microbial samplers or two Reuter-Centrifugal samplers, and the total number of airborne aerobic bacteria and concentrations of airborne *Escherichia coli* were measured. The concentrations of indoor airborne aerobic bacteria of the rabbitry were from 6.1×10^3 to 2.0×10^4 CFU/m^3 air, and of the hen house were from 9.7×10^4 to 4.9×10^5 CFU/m^3 air. The concentrations of airborne *Escherichia coli* of the rabbitry and the hen house were from 3.5×10^2 to 4.9×10^2 CFU/m^3 air and from 9.5×10^2 to 1.3×10^3 CFU/m^3 air, respectively. The concentrations of outdoor airborne aerobic bacteria and airborne *Escherichia coli* declined rapidly from 10 m to 800 m. The indoor airborne concentrations of aerobic bacteria in both hen house and rabbitry were significantly higher than those of outdoor (downwind 10, 50, 100, 200, 400, 600, 800 m) ($p<0.01$). The total numbers of airborne aerobic microorganisms of outdoor (downwind 10, 50, 100, 200 m) samples were significantly higher ($p<0.01$) than those of windward samples. Resistance against antibiotics isolated *Escherichia coli* strains from indoor and outdoor air samples were also analyzed. Twelve antibiotics were selected: norfloxacin (NOR), cefoperazone (CFP), chloromycetin (CMP), complex sulfanilamide (SXT), gentamycin (GEN), streptomycin (STR), tetracycline (TET), rifampicin (RIF), erythromycin (ERY), penicillin (P-G), tobramycin (TOB) and furantoin (Ni). The results showed all *Escherichia coli* strains isolated from indoor and outdoor (downwind 10, 50, 100, 200 m) air samples in the rabbitry, feed and feces resisted against RIF and P-G, while they were sensitive to TOB, GEN and Ni. The strains from indoor and outdoor (downwind 10, 50, 100, 200 m) air samples in the hen house, feed and feces were sensitive to CFP, GEN and Ni, and resisted against RIF, ERY and P-G. This could be concluded that indoor microbial aerosols include antibiotic resistance bacteria could transmit to surroundings by air exchanging and cause microbiological contamination of the air as well as epidemic transmission.

Keywords: animal house surroundings; microbial aerosol; antibiotic resistance bacteria; spreading; microbial pollution

INTRODUCTION

Aerosol is a system of solid or liquid particles diffused in air. Particles of microbial aerosol are microorganisms. Microorganisms resulting from animal dander, faecal matter, feeds and water can accumulated and form microbial aerosols. Modern agricultural methods have changed the way animals are raised. More and more antibiotics are used to prevent and cure animal diseases, and that results in the producing of more antibiotic resistance bacteria. Microbial aerosols produced from animal houses can be transmitted to environment via air exchanging and cause microbiological contamination, diffusion of pathogens and antibiotic resistance bacteria[1]. This compromises the health of inhabitants of nearby societies.

Escherichia coli are a common flora of worm-blooded animals, and also pathogens or selective pathogens. This study measured the concentrations of airborne *Escherichia coli* and analyzed their resistance against antibiotics in order to find the mode of the diffusion of antibiotic resistant microbial aerosols.

MATERIALS AND METHODS

Environment of the hen house and the rabbitry

The studied rabbitry is partially closed with many open windows. The farm has four of these rabbitries that raised breeding rabbits and broiler rabbits. The rabbitries are cleaned everyday.
The hen house is built in closed style with a mechanical ventilation system to help maintain air exchange and two thirds of the ground is covered with wood slats. The farm has seven of these hen houses which feed 5000 hens each.

Sampling and analysis

Airborne microorganism samples of the rabbitry were collected from indoor and outdoor air (down wind 10, 50, 100, 200 m and windward 100 m as a comparison sample) at two days in March 2005 and May 2005. Air samples of the hen house were collected from indoor and outdoor air (down wind 10, 50, 100, 200, 400, 800 m and windward 100 m as a comparison sample) at two days in October 2005 and November 2005. Two 6-stage Andersen samplers[2] and two Reuter-Centrifugal samplers were used to collect the samples.

The samplers located 80 cm above the ground and operated for 1 to 8 min. The 6-stage Andersen samplers operated at 28.3 L/min and the Reuter-Centrifugal samplers operated at 40 L/min. The samplers were preautoclaved in the laboratory and disinfected by 70% ethanol-immersed cotton balls between each sampling.

Airborne microorganisms were collected onto 20 ml of agar with 5% sheep blood in 90-mm-diameter plates. The samples were incubated at 37°C for 24 h. Then the number of grown colonies was counted. The positive hole method was applied to AMS samples for correction[2].

Feed and feces sampled from different site in the stalls at random.

Isolation and identification of airborne *Escherichia coli*

Short gram-negative bacilli on the blood-agar plates were streaked in MacConky's (MAC) plates and incubated at 37°C for 24 h. Then pink colonies on MAC were applied and identified by using the API-20 E and API-20 NE system (BioMerieux).

Isolation and identification of *Escherichia coli* from feed and feces

0.5 g of feces or feeds sample were dissolved in 4.5 ml sterilized broth and incubated at 37°C for 24 h. Streaked in MAC plates and incubated at 37°C for 24 h. Then pink colonies were applied and identified by using the API-20 E and API-20 NE system (BioMerieux).

Antimicrobial susceptibility testing

Twenty *E. coli* isolates from each sample site, feed and feces at a day were used to test antimicrobial susceptibility by Kirby-Bauer method (only 1 strain was isolated at windward 100 m and downwind 400, 800 m outside the hen house respectively, so not tested). Twelve antibiotics were selected: norfloxacin (NOR), cefoperazone (CFP), chloromycetin (CMP), complex sulfanilamide (SXT), gentamycin (GEN), streptomycin (STR), tetracycline (TET), rifampicin (RIF), erythromycin (ERY), penicillin (P-G), tobramycin (TOB) and furantoin (Ni). The results were estimated by standards of CLSI[3].

RESULTS

Concentrations of airborne microorganisms of the rabbitry

The concentrations of airborne aerobic bacteria of the rabbitry were from 6.1×10^3 to 2.0×10^4 CFU/m^3 indoor, from 2.4×10^2 to 5.0×10^2 CFU/m^3 at windward 100 m and from 2.9×10^2 to 7.1×10^3 CFU/m^3 at 10 to 200 m downwind. The number of airborne *E. coli* ranged from 3.5×10^2 to 4.9×10^2 CFU/m^3 indoor, from 2.5×10 to 7.4×10 CFU/m^3 at 10 to 200 m downwind and no *E. coli* was isolated at windward 100 m.

Statistic results showed the number of indoor airborne aerobic bacteria was higher than that of downwind 10 m ($P<0.05$) and significantly higher than those of downwind 50, 100, 200 m ($P<0.01$). The concentration of indoor airborne *E. coli* was significantly higher than those of downwind 10, 50, 100, 200 m ($P<0.01$). The concentrations of airborne aerobic bacteria of indoor and downwind 10, 50, 100, 200 m were significantly higher than that of windward 100 m ($P<0.01$). The number of airborne bacteria of downwind 10 m was significantly higher than that of downwind 50 m ($P<0.01$). Analysis showed no significant difference among the airborne concentrations of microbe of downwind 50, 100, 200 m ($P>0.05$). Bioaerosol concentrations of the rabbitry and its surroundings were given in table 1.

Table 1. Concentrations of airborne microorganisms measured in the rabbitry (CFU/m^3)

Sampling place	n	Culturable bacteria				*Escherichia coli*		
		Max.	Min.	Median		Max.	Min.	Median
Indoor	8	2.0×10^4	6.1×10^3	9.5×10^3		4.9×10^2	3.5×10^2	4.1×10^2
Downwind 10 m	8	7.1×10^3	2.6×10^3	5.6×10^3		7.4×10	4.8×10	6.9×10
Downwind 50 m	8	9.6×10^2	4.7×10^2	5.6×10^2		6.4×10	5.0×10	5.9×10
Downwind 100 m	8	8.6×10^2	4.7×10^2	5.9×10^2		5.0×10	2.9×10	4.3×10
Downwind 200 m	8	6.8×10^2	2.9×10^2	4.2×10^2		4.8×10	2.5×10	3.4×10
Windward 100 m	8	5.0×10^2	2.4×10^2	3.5×10^2		0	0	0

n: number of samples, Max.: Maximum, Min.: Minimum, CFU: colony forming units.

Concentrations of airborne microorganisms of the hen house

As described in table 2, the concentrations of indoor airborne aerobic bacteria and culturable *E. coli* of the hen house were from 9.7×10^4 to 4.9×10^5 CFU/m^3 and from 9.5×10^2 to 1.3×10^3 CFU/m^3, respectively. The concentrations of outdoor airborne aerobic bacteria and airborne *E. coli* from 10 m to 800 m were ranged from 5.8×10^2 to 4.1×10^4 CFU/m^3 and from 0 to 4.2×10^2 CFU/m^3, respectively. Only 1 strain of *E. coli* was isolated from downwind 400 or 800 m air samples, and no *E. coli* was isolated from downwind 600 m. The airborne concentrations of aerobic microbe of windward 100 m were from 8.0×10^2 to 1.1×10^3 CFU/m^3, and only 1 strain of *E. coli* was isolated.

Statistic results showed the number of indoor airborne aerobic bacteria was significantly higher than that of downwind 10, 50, 100, 200, 400, 600, 800 m ($P<0.01$). The concentration of indoor airborne *E. coli* was significantly higher than that of downwind 10, 50, 100, 200 m ($P<0.01$). The concentrations of airborne aerobic bacteria of indoor and downwind 10, 50, 100, 200 m were significantly higher than that of windward 100 ($P<0.01$). The number of airborne bacteria of downwind 10, 50, 100, 200 m were significantly higher than those of downwind 400, 600, 800 m ($P<0.01$). Analysis showed no significant difference among the airborne concentrations of bacteria of windward 100 m and downwind 400, 600, 800 m ($P>0.05$).

Table 2. Concentrations of airborne microorganisms measured in the hen houses (CFU/m^3)

Sampling place	n	Culturable bacteria				*Escherichia coli*		
		Max.	Min.	Median		Max.	Min.	Median
Indoor	15	4.9×10^5	9.7×10^4	2.7×10^5		1.3×10^3	9.5×10^2	1.1×10^3
Downwind 10 m	15	4.1×10^4	3.6×10^4	3.8×10^4		4.2×10^2	3.2×10^2	3.7×10^2
Downwind 50 m	15	3.7×10^3	3.0×10^3	3.3×10^3		2.6×10^2	1.7×10^2	2.1×10^2
Downwind 100 m	15	2.8×10^3	2.1×10^3	2.5×10^3		6.1×10	4.1×10	4.9×10
Downwind 200 m	15	3.8×10^3	1.8×10^3	2.6×10^3		4.6×10	3.5×10	4.1×10
Downwind 400 m	15	1.5×10^3	5.8×10^2	1.0×10^3		4.2	0	0
Downwind 600 m	15	9.1×10^2	6.0×10^2	8.0×10^2		0	0	0
Downwind 800 m	15	8.1×10^2	6.0×10^2	7.4×10^2		4.2	0	0
Windward 100 m	15	1.1×10^3	8.0×10^2	9.0×10^2		4.2	0	0

n: number of samples, Max.: Maximum, Min.: Minimum, CFU: colony forming units.

Results of antimicrobial susceptibility testing

All strains isolated from indoor and outdoor air samples in the rabbitry, feed and feces resisted against RIF and P-G, and some strains resisted against NOR, CFP, CMP, SXT, STR, TET and ERY (table 3).

Table 3. Results of antimicrobial susceptibility testing of *E. coli* from the rabbitry (%[#])

Sampling place	n	NOR	CFP	CMP	SXT	GEN	STR	TET	RIF	ERY	P-G	TOB	Ni
Indoor	20	15	15	15	70	0	30	70	100	80	100	0	0
Downwind 10 m	20	15	0	15	50	0	25	50	100	90	100	0	0
Downwind 50 m	20	15	0	15	35	0	30	45	100	100	100	0	0
Downwind 100 m	20	20	0	15	45	0	15	55	100	100	100	0	0
Downwind 200 m	20	10	0	0	20	0	20	50	100	100	100	0	0
Feces	20	20	20	15	75	0	50	70	100	100	100	0	0
Feed	20	10	0	10	20	0	35	50	100	100	100	0	0

#: percentage of resistant isolates to total tested isolates.
N: number of samples

The strains from indoor and outdoor air samples in the hen house, feed and feces resisted against RIF, ERY and P-G., and some strains resisted against NOR, CFP, CMP, SXT, STR and TET (table 4).

DISCUSSION

The level of airborne bacteria and *E. coli* in this study were different from those in many published reports[4,5,6,7]. It is reasonable to attribute to the structure of the stalls, feeding method, animal density and cleaning practice and frequency. Bioaerosol in stalls mostly come from the animals, microorganisms resulting from animal faecal matter, dander and feed materials are easily accumulated and aerosolized[8]. Proper animal density, ventilation and cleaning are effective measures to reduce the concentrations of airborne microorganisms.

The concentrations of outdoor airborne aerobic bacteria and airborne *E. coli* were lower than that of indoor and declined rapidly from downwind 10 m to 800 m. This could be concluded that indoor microbial aerosols could transmit to surroundings by air exchanging and form a higher concentration of bacteria near the farm. This microbiological contamination compromised the health of inhabitants of nearby societies. Pathogenic *E. coli* could cause hominine diarrhoea, hemorrhagic colitis and urogenital system inflammation.

Table 4 Results of antimicrobial susceptibility testing of *E. coli* from the hen houses(%[#])

Sampling place	n	NOR	CFP	CMP	SXT	GEN	STR	TET	RIF	ERY	P-G	TOB	Ni
Indoor	20	55	0	0	55	0	45	80	100	100	100	0	10
Downwind 10 m	20	0	0	0	50	0	0	50	100	100	100	0	0
Downwind 50 m	20	0	0	0	40	0	0	45	100	100	100	0	0
Downwind 100 m	20	0	0	15	35	0	30	50	100	100	100	0	0
Downwind 200 m	20	0	0	0	0	0	40	50	100	100	100	0	0
Feces	20	30	20	0	70	0	40	70	100	100	100	0	0
Feed	20	20	0	0	25	0	50	50	100	100	100	0	0

#: percentage of resistant isolates to total tested isolates.
N: number of samples

As shown in table 3, the percentages that the *E. coli* strains isolated from rabbit feces resisted against NOR, SXT, CFP, STR, ERY was 20%, 75%, 50%, 50%, 100%, respectively. These were higher than those of indoor airborne *E. coli* strains: 15%, 70%, 30%, 30%, 80%. All *E. coli* strains

from indoor air samples and feces were resisted against P-G and RIF, and the percentages they resisted against TET and CMP were equal. Similar results were got from the *E. coli* strains from the hen house and hen feces. These accorded with the routine usage of medicine of the farms which were obtained on the sampling days. *E. coli* strains resisted against medicines that used frequently and were sensitive to those rarely used. This could be concluded that airborne antibiotic resistant bacteria come from animal feces.

Results of antimicrobial susceptibility testing showed *E. coli* strains isolated from the rabbitry surroundings resisted against P-G and RIF, the same as *E. coli* strains from indoor air samples. *E. coli* strains from the hen house and its surroundings resisted against RIF, ERY and P-G. All tested isolates had similar ratio of resistance against other antibiotics. This showed outdoor airborne antibiotic resistant bacteria come from the stalls by air exchanging.

The percentage that airborne *E. coli* strains isolated from indoor air samples of the rabbitry resisted against NOR was lower than that of downwind 100 m outside the rabbitry. The percentage that indoor airborne *E. coli* strains isolated from the rabbitry resisted against ERY was lower than those of downwind 10, 50, 100, 200 m. This indicated that downwind airborne antibiotic resistant bacteria had other resources besides the rabbitry, such as nearby societies and other farms. No such results had been found from the studied hen house probably because there were no farms near it and 1000 m away from the nearest village.

ACKNOWLEDGEMENTS

This study was financially supported by NSFC grant (30571381) “The spreading mode of microorganism aerosol in animal farms”.

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