

The Association between Daily Contact, Working Period and Biosecurity to Colonization of Extended-Spectrum Beta Lactamase-Producing *Escherichia coli* of Poultry Workers

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Abstract: Extended-spectrum beta lactamase producing *Escherichia coli* (ESBL-Ec) colonization in poultry worker is one prominent of zoonotic problem, in which of it, daily contact, working period and biosecurity play role. This study aimed to identify association between daily contact, working period and biosecurity to ESBL-Ec' colonization. Rectal swab was applied to poultry workers to identify phenotypic ESBL-Ec. Duration of contact, working period and biosecurity parameter data was obtained through structured questionnaire. A number of 51 poultry workers with a mean age of 42.7 years, and 54.9% are male. Nineteen poultry workers (37.3%) was tested positive for ESBL-Ec. Daily contact of ≥ 2 hours is not significant ($p = 0.291$; 95% CI) while working period of ≥ 3 years is significant ($p = 0.024$; 95% CI) to ESBL-Ec' colonization. Twenty-one biosecurity parameters were measured, with 3 parameters significant association: sick chicken isolation ($p = 0.022$), existence of quarantine shed ($p = 0.018$) and past biosecurity training ($p = 0.047$). In conclusion, working period ≥ 3 years, sick chicken isolation, existence of quarantine shed and past biosecurity training is associated with ESBL-Ec' colonization to farmer.

Keywords: Biosecurity; ESBL-Ec colonization; Daily contact; Working period

Introduction

Extended-spectrum beta-lactamase producing *E. coli* (ESBL-Ec) is one type of multi drugs resistance *E. coli* that is difficult to treat, worse clinical outcome, higher mortality rate, extended hospital stay compared to non ESBL pathogens (Alsamawi et al., 2022). Colonization of ESBL-Ec is potential threat of having severe infection in the future (Husna et al., 2023). This potential threat could be detected in a healthy human and the risk of acquisition could be analyzed statistically and or molecular relatedness. ESBL-Ec surveillance in livestock farms showed that chicken farm (8/9), followed by pig

(5/10) and cattle farm (3/10) (Ludden et al., 2019). In Indonesia, the prevalence of ESBL-Ec from broiler chicken is around 67.1% (Puspandari et al., 2021), indicate that chicken farm has a higher risk of ESBL-Ec colonization and how its' workers is affected.

Poultry worker is a specific population with hazard risk to poultry, especially in poultry farm that is use antibiotic as growth promoter. This hazard risk related to poultry consist of microbiological/chemical contamination to air, water and soil, impact to the health of worker and wider society (Gržinić et al., 2023). ESBL-Ec transmission is biological hazard that is included as zoonotic diseases. Irrational use of antibiotics is one of

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contributing factors in the spread of multi drugs resistance organisms (Machowska et al., 2019). In recent studies ESBL-Ec, molecular similarity between chicken and humans (Badr et al., 2022). Nigerian study indicated higher prevalence of ESBL-Ec in poultry farms was found to worker with higher contact with poultry (Aworh et al., 2019). Those studies indicate that contact with chicken could be the main source of ESBL-Ec colonization to a healthy worker.

Duration of daily contact with chicken and period of working are increased occupational exposure and risk accumulation (Gržinić et al., 2023). Biosecurity is a preventive measure to prevent transmission of infectious diseases between human, human to animal, vice versa (Butucel et al., 2022). This preventive measure means holistic approach based on open/ closed herd, visitor management, and visitors' clothing, footwear, and equipment (yes/no questions) were discussed at the visits to this farm (Denis-Robichaud et al., 2020).

Method

Subjects were randomly selected to intensive farm poultry workers in Teruwai village. All subjects who met inclusion criteria: minimum 18 years old, healthy, willing to be interviewed for 20 minutes questionnaire and brief training on self-obtained of swab samples (Figure 1).

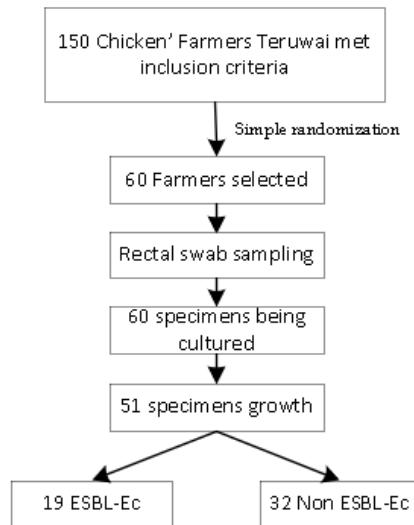


Figure 1. Research flow

Swab samples were cultured in Biomedicine Laboratory, NTB provincial hospital, using EMB agar and MacConkey agar to specifically grow *E. coli* species, all presumptive *E. coli* were proceeded with IMViC test. Phenotypic test of Extended-Spectrum Beta Lactamase (ESBL) namely double disc synergy test as described by CLSI (2019).

Result and Discussion

The subjects in this study were 51 subjects, of which 28 subjects (54.9%) were male, and 23 (45.1%) were female. The average age of the subjects of this study was 42.7 years. Based on gender and age, study by Nguyen et al. (2019) found that male farmer had a larger compared to female farmer with average age 46 years, that is equivalent to current study. In South Asia it is common that women take part in farming between 60-89% (Asadullah, 2020). Based on our observations, the local wisdom that is implicitly stated "it is obligated that is women is actively both household and farming activities".

The number of ESBL-Ec colonization in farmer was 19/51 (37.3%) is lower compared to Bangladesh study (Rousham et al., 2021) 67.5%, higher than Vietnam 35.2% (Nguyen et al., 2019).

Table 1. The association between duration of daily contact and working period to ESBL-Ec colonization

		ESBL-Ec		p value 95% CI
		Positive	Negative	
Duration of daily contact	≥ 2 h/d	10 (45.5%)	12 (54.5%)	0.291
	<2 h/d	9 (31%)	20 (69%)	
Working period	≥ 3 years	15 (50%)	15 (50%)	0.024
	< 3 years	4 (19%)	17 (81%)	

Daily contact duration parameters divided into: two hours or more per day and less than two hours per day, working period parameters divided into: three years or more and less than three years. Based on the daily contact duration parameters, 22 subjects (43.1%) worked for 2 hours or more per day and 29 subjects (56.9%) worked for less than 2 hours per day. The duration of daily contact was the first parameter analyzed in this study, where these parameters were grouped into 2 categories, namely duration of daily contact for ≥ 2 hours/day and < 2 hours/day. Based on the results of the interview, the difference in daily contact duration on the subjects of this study was caused by the number of the chickens and cages that he/she took care of. Most poultry workers who had duration of daily contact for ≥ 2 hours/day had higher chicken densities and more cages.

ESBL-Ec colonization was higher in study subjects who had duration of daily contact for ≥ 2 hours/day compared to < 2 hours/day (45.5% vs 31%), even this difference was not statistically significant ($p = 0.291$; 95% CI).

The route of ESBL-Ec transmission in chicken farm is complex, transmission from chickens to humans can occur horizontally, either directly or indirectly. Direct

ESBL-Ec transmission is possible through close contact with chickens. Activities related to close contact with chickens are feeding and drinking, controlling the temperature of the house, regulating air circulation, controlling faecal conditions, and controlling the condition of chickens (Khalifa et al., 2024).

However, in this study it was found that the duration of daily contact did not have a significant relationship. This different result could be due to the fact that ESBL-Ec colonization in chicken farmers is not only related to the duration of daily contact of the poultry workers but is multifactorial. Based on research conducted by Wu et al. (2019), there are several other factors that are significantly related to the incidence of ESBL in chicken farmers, namely a history of diabetes and skin disease in farmers, the amount of activity carried out in the chicken coop, and the presence of a bathroom (shower) in chicken coop.

Based on working period, 30 subjects (58.8%) worked for ≥ 3 years, and 21 subjects (41.2%) worked for <3 years. There were 19 subjects (37.3%) poultry workers ESBL-Ec positive. Based on the duration of daily contact of the study subjects, in the group with daily contact duration 2 hours or more per day there were 10 subjects (45.5%) with positive ESBL-Ec and 12 subjects (54.5%) no ESBL-Ec. Meanwhile, in the group with daily contact duration < 2 hours there were 9 subjects (31%) with ESBL-Ec, and 20 subjects (69%) no ESBL-Ec. On the other hand, based on the working period of the research subject, in the group with a working period 3 years or more there are 15 subjects (50%) with ESBL-Ec, and 15 subjects (50%) without ESBL-Ec. In the group with working period less than 3 years there are 4 subjects (19%) with ESBL-Ec and 17 subjects (81%) without ESBL-Ec. Chi-square test showed the difference between 2 hours or more per day and less than 2 hours per day was not statistically significant ($p = 0.291$; 95% CI). Working period parameters, showed that positive ESBL-Ec colonization results of 3 years or more compared to less than 3 years had a significant difference ($p = 0.024$; 95% CI). In this study, the relationship between working period and the incidence of ESBL-Ec colonization in chicken poultry workers in Teruwai was also analysed. In contrast to the duration of daily contact parameter, the results showed that ESBL-Ec colonization was higher in study subjects with a working period 3 years or more compared to < 3 years (50% vs 19%) with a statistically significant difference ($p = 0.024$; 95% CI). Study subjects with a working duration of 3 years or more had a greater tendency to colonize ESBL-Ec than study subjects with a working period less than 3 years. This result is in accordance with the research of (Aworh et al. 2019) which found that occupational exposure is a risk factor

that is significantly related to the occurrence of ESBL-Ec colonization in chicken poultry workers.

Close contact to farm is believed to had higher risk of ESBL-Ec' colonization but duration of daily contact was not the case, probably another type of close contact is how daily contact is accumulated, or working period. One qualify study mentioned about colonization is concordance with antibiotic exposure (Lewis et al., 2022), which is typical for chicken farmer; antibiotic use in intensive chicken farming as growth promoter.

The colonization of ESBL-Ec in humans can have an impact on health in the future. *E. coli* is the most common bacterial cause of urinary tract infection (UTI) in the community, followed by intra-abdominal and soft tissue infections such as diarrhea, sepsis, acute enteritis, hemorrhagic colitis, and neonatal meningitis to sepsis. Therefore, resistance to antimicrobials used in the treatment of *E. coli* infection is a problem in the treatment of this occurring infection (Nguyen et al., 2019). Furthermore, ESBL-Ec also has a broad spectrum of infections and has higher morbidity and mortality (Shamsrizi et al., 2020).

Biosecurity practice in this study assessed based on designated area of the isolation principles, considered as high risk (red zone), moderate risk (yellow zone) and low risk (green zone) (Kementan & FAO, 2020).

Table 2. Biosecurity practice parameter and ESBL-Ec colonization. NA = Not Applicable, PPE = Personal Protective Equipment

Parameter	ESBL-Ec	Non <i>p</i> value		ESBL-Ec 95% CI
		Open	Closed	
Type of farm house type	Open	22	72	0.000
	Closed	48	13	
Separation between farm house and workers' house	<500 metres	18	29	
	≥ 500 metres	1	3	1.000
Span between farm house to main street	<500 metres	16	27	
	≥ 500 metres	3	5	1.000
Space between farm house	<10 metres	16	28	
	≥ 10 metres	3	4	1.000
Parking area	Yes	12	22	
	No parking area	7	10	0.763
Farm house structure	Permanent	9	7	
	Semi-Permanent	10	25	0.070
Quarantine shed	Yes	3	16	
	No	16	16	0.018
Number of workers in each farm house	One	12	13	
	More than one	7	19	0.153

Parameter		ESBL-Ec	Non <i>p</i> value	
			ESBL-Ec	95% CI
Cleaning water feed container	Routine	6	16	0.250
	Not routine	13	16	
Chicken manure disposal	Routine	4	11	0.360
	Not routine	15	21	
Foot and hand disinfect	Yes	0	3	0.285
	No	19	29	
Tools' cleaning	Yes	10	16	1.000
	No	9	16	
Farm tools use	Trans-zone	2	6	0.649
	Not trans-zone	17	26	
Sick/decease chicken monitoring	Routine	15	23	0.743
	Not routine	4	9	
Boot/ foot wear use	Not trans-zone	15	27	0.711
	Trans-zone	4	5	
Sick chicken isolation	Yes	14	31	0.022
	No	5	1	
Biosecurity training	Yes	5	18	0.047
	No	14	14	
Quarantine shed	Yes	3	16	0.018
	No	16	16	
PPE use before farm house entry	Yes	0	0	NA
	No	19	32	
PPE use on farm house cleaning	Yes	0	0	NA
	No	19	32	
PPE use on sick/decease chicken transfer	Yes	0	0	NA
	No	19	32	
Hand hygiene before and after enter farm house	Yes	0	0	NA
	No	19	32	

There were 21 biosecurity practice parameters that is assessed its association to ESBL-Ec colonization in farmer workers. There are 7 parameters of structural biosecurity include: type of farm house, Separation between farm house and workers' house, Span between farm house to main street, Space between farm house, existence of parking area, structure of farm house and existence of quarantine shed. Parameter of operational biosecurity: Number of workers in each farm house, Cleaning water feed container, Chicken manure disposal, Foot and hand disinfect, Tools' cleaning, Farm tools use, Sick/decease chicken monitoring, Sick chicken isolation, Biosecurity training, PPE use before farm house entry, PPE use on farm house cleaning, PPE use on sick/decease chicken transfer, Hand hygiene before

and after enter farm house. Three biosecurity parameters were significant ($p < 0.005$): procedure in isolation of sick chicken ($p = 0.022$), existing of quarantine shed ($p = 0.018$) and having experience on biosecurity training ($p = 0.047$).

Biosecurity practice in livestock refers to the actions taken to prevent and control the emergence and spread of infectious diseases (Mappanganro et al., 2019). The application biosecurity there are structural biosecurity; construction of the farm house, maintenance of farm house and operational biosecurity; procedures, practice and policy that carefully followed by poultry workers (USDA, 2020). In this research, biosecurity practice that is significant association to ESBL-Ec colonization in poultry workers were: procedure in isolation of sick chicken ($p = 0.022$), existing of quarantine shed ($p = 0.018$) and having experienced on biosecurity training ($p = 0.047$).

Isolation means keeping chickens away from people, vehicles and objects that can carry pathogens, and create an environment in which chickens are protected from carriers of pathogenic bacteria (people, other animals, air and water). Isolation is a major component of biosecurity. Procedure in isolation of sick chicken developed based on self-observation made by poultry workers upon returning from biosecurity training or after farming counsellor education. Sign and symptoms of sick chicken that Teruwai' poultry workers recognized is immobility, bluish chicken comb crown, nasal and ocular discharge and snoring. Isolation not only known in separation healthy chicken from the sick ones, also never run a mix species of flocks, build the farm house and select a location, and minimizing visitors. Interestingly, there is general awareness among majority subjects in keeping visitor as minimum as possible, not because of biosecurity, but because of stress of the chicken facing strangers in the farm house.

Isolation of sick chicken needs a special place like quarantine shed. Isolation is the separation of animals in a controlled environment or place to prevent disease agents from appearing (Mappanganro et al., 2019). In this study, the existence of quarantine shed tends to increase monitoring sick/decease poultry activity, thus had a significant association with ESBL-Ec colonization in poultry workers. In addition, biosecurity training among poultry workers has a significant association to ESBL-Ec colonization. Based on informations from locals, after in 2014 Governor declare that is Teruwai as 'poultry village', periodically Farming Biro would monitor the farm, giving pestisides, DOC distribution, training on biosecurity, training on egg production or meat production. After training follow up, occasionally done with free distribution of vitamins, even DOC.

Furthermore, limitation of this study is the number of samples that is limited, possibly of under detection of *Escherichia coli* due to self-obtain of swab samples by subjects, and no cloacal swab samples from the chickens.

Conclusion

Working period has a significant relationship to ESBL-Ec colonization of intensive chicken farmers in Teruwai Village, Central Lombok. The ESBL-Ec colonization rate was higher in study subjects with daily contact duration \geq 2 hours / day, but this difference was not statistically significant. Biosecurity practice in isolation of sick chicken, existence of quarantine shed and biosecurity training was statistically significant in poultry workers' ESBL-Ec colonization.

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Author Contributions

Conceptualization, E.H.W.; methodology, E.H.W. and M.A.; writing – original draft preparation, E.H.W. and S.A.E.J.

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Conflicts of Interest

The authors declare no conflict of interest.

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