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Isolation of Microorganisms Using Chromogenic Orientation Media from Healthy Gastrointestine Tract (GIT) Chicken

Rozila Alias[1,2*], Thivyah Karunageran[2], Siti Nurfatehah Kamal[2], Faizal Riza Abu Hasan[5], Siti Hasmah Mohtar[3], Yusmi Mohd Yunus[3], Mohd Nizam Lani[4], Norazah Mohammad Nawawi[1,2] and Abdul Latif Ibrahim[2]

[1]*Centre for Foundation and General Studies, Universiti Selangor, Bestari Jaya, Kuala Selangor, Selangor.*
[2]*Institute of Bio-IT Selangor, Universiti Selangor, Jalan Zirkon, A/7A, Seksyen 7, 40000 Shah Alam, Selangor.*
[3]*Faculty of Engineering and Life Sciences, Universiti Selangor, Bestari Jaya, Kuala Selangor, Selangor.*
[4]*Faculty of Fishery and Food Science, Universiti Malaysia Terengganu, Kuala Nerus, Kuala Terengganu, Terengganu*
[5]*Geneflux Biosciences Sdn Bhd., G-1, Menara KLH, Bandar Puchong Jaya, 47100 Puchong, Selangor.*

*Correspondence Email: rozila@unisel.edu.my

Abstract: Sustainable chicken meat production is an important to provide a safe and good quality protein sources for human as foods and nutritions. The gastrointestinal tract (GIT) of chickens harbors a diverse and a complex microbiota that plays a vital role in digestion and absorption of nutrients, immune system development and pathogen exclusion. But however, the integrity, functionality and health of chicken guts depends on many factors, including environments, feed and the GIT microbiota. The symbiotic interactions between host and microorganisms is a fundamental to improve chicken health and production. Recently, many research studies were carried out on the chicken GIT microbiota relied on new techniques either molecular or microbiological study. A new chromogenic plate medium, CHROMagar Orientation, was used in this study to differentiate and presumptive identification of gram-negative bacilli and enterococcus species by differentiating color on agar plates. The aim of this study is to isolate microbes in five parts of GIT healthy chickens. Two local chicken farms at Kuala Pilah, Negeri Sembian; one local chicken farm and two broiler chicken farms at Meru, Kuang and Batang Kali, Selangor. Each farm was collected in duplicate sampling of Healthy GIT chicken. Then the GIT was divided into five parts and microorganisms were isolated using a chromogenic agar plate. After the 37^{o}C overnight incubation, colonies on the plates were grown and counted. Colony forming unit (CFU) were calculated in each parts based on three basic colour (blue-torquis, pinkish and cream-white). These three colour presented 12 bacteria species such as *Klebsiella, Citrobacter, Enterococcus, S.marcencent, S. agalactiae, Escherichia coli, S. saprophyticus, Pseudomonas, Acinetobacter, S. aureus, S.epidermis* and *Providensia*. From the results, two local farms (A and D) and one broiler farms (E) gave a similar results with value of Log^{10}CFU/ml within range 4.2626 to 4.1200. Then another two farms (B and C) gave a value of Log^{10} CFU/ml which are 0.9755 (local chicken farm) and 1.124 (broiler chicken farm). Thus, this understanding of value reported that these two farms have their chickens with low number of percentage microorganism in GIT's chicken. This will reflect that the chickens are healthier than other farms. The chromogenic medium was easier found to be facilitating visual by the colouring detection of mixed bacterial species isolates. Further, there is a need for better understanding of the chicken GIT function and microbiology study that will provide us new opportunities for the improvement of chicken health, meats and egg productions.

Keywords: chicken, GIT microbiota, Chromogenic media, pathogens

1. Introduction

A healthy gastrointestinal tract (GIT) is essential for efficient conversion of feed into its basic component for optimal nutrient absorption. The GIT has its function for digestive, absorptive, metabolic, immunological and endocrinological. Over the past two decades, this GIT issue has gained more interest in poultry production due to increasing demands for economic efficiency, animal welfare, food safety, reduction in environmental impacts, and a ban on or avoidance of growth promotant antibiotic use

(Edgar, 2019). Chromogenic agar offers a faster approach for identification (ID) of pathogens from mixed cultures such as urine cultures. The present study aims to presumptive identification bacteria isolated from GIT based on color discrimination and colony morphology using Chromagar orientation plate.

2. Materials and Methods

Isolations of microorganisms

Five chicken's farms were selected randomly and triplicate GIT of healthy chickens were taken and proceed to isolate the microorganisms onto Chromogenic agar plates (CHROMagar Orientation plates). A serial dilution 10^{-1} until 10^{-10} was prepared and GIT was divided into five parts which are trakea, duodenum, small intestine, colon and caesum (Figure 1). Then, the samples were spread onto chromogenic agar and incubated at 35ºC to 37ºC for within 16 to 24hr. Colonies with various colors growth on agar and counted using Scan®100 (Interscience) colony counter. The colour of colonies were appeared according to manufactured instruction (Table 1) of expected species and recorded. Colony forming unit (CFU) value were calculated as in Table 2.

$$\text{Colony forming unit (CFU)/ml} = \frac{\text{number of colonies x dilution factor}}{\text{volume on plates (ml)}}$$

Table 1: Guidelines for Presumptive Identification Based on Different Colony Colors

Bacteria species Group	Presumptive Bacteria species	Colors and colony morphology shape
KECS group (Blue-torquise)	*Klebsiella*	Medium-metalic-torquise blue to dark blue colonies
	Citrobacter	Blue-purple colonies
	Enterococcus	Blue-green small colonies
	S.marcencent	Aqua Blue
	S. agalactiae	Light blue-green to light blue, pinpoint to small colonies, with or without halos
Ec (Pinkish)	*Escherichia coli*	Dark rose to pink, transparent colonies, medium to large size, with or without halos in the surrounding medium
	S. saprophyticus	Light pink to rose, small opaque colonies with or without halos
PAS group (cream- white)	*Pseudomonas, Acinetobacter S. aureus, S.epidermis, Providensia*	Cream to yellow or clearance, small colonies

Storage cultures

After pure cultures were confimed, all the isolates were stored into the bead storage and keep at -20ºC freezer refrigerator until used.

3. Results and Discussion

Appearance of colonies on CHROMagar Orientation plates.
Colonies were grown on CHROMagar Orientation plates after 16 to 24 h incubation. There was a consistent color reaction observed for some species or genus, with discrepancies summarized in Table 1.

Calculation for Colony Forming Unit (CFU)
Colony forming unit (CFU/ml) value was performed. The number of each colony with three basic colors (blue, pink and cream) were recorded to facilitate the identification of microorganism species on chromogenic plates. CFU/ml were calculated in each different GIT parts and overall CFU/ml and Log^{10} CFU/ml as stated in Table 2.

Table 1: Colony count of colonies grow on Chromogenic agar based on three basis colours.

Farm	Location	Chicken	GIT parts	No. of Colonies growth on Chromogenic agar plate			
				Blue-Torquios	Pinkish	Cream - white	Total bacteria
A	Kuala Pilah, Negeri Sembilan	Local	Esophagus	26	17	23	66
			Duodenum	96	123	16	235
			Small intestine	80	32	11	123
			Large intestine	18	9	1	28
			Cloaca	54	0	20	74
B	Kuala Pilah, Negeri Sembilan	Local	Esophagus	2	2	0	4
			Duodenum	4	1	24	29
			Small intestine	48	20	0	68
			Large intestine	81	43	0	124
			Cloaca	13	9	19	41
C	Meru, Klang, Selangor	Broiler	Esophagus	7	7	10	24
			Duodenum	0	2	0	2
			Small intestine	6	5	0	11
			Large intestine	10	17	0	27
			Cloaca	18	55	2	75
D	Kapar, Klang, Selangor	Local	Esophagus	196	31	23	250
			Duodenum	57	39	9	105
			Small intestine	140	10	0	150

			Large intestine	206	28	5	239
			Cloaca	36	38	6	80
E	Kuang, Rawang, Selangor	Broiler	Esophagus	15	23	19	57
			Duodenum	28	33	67	128
			Small intestine	42	45	4	91
			Large intestine	44	51	16	111
			Cloaca	139	99	9	247

Table 2: Estimation of Overall Average of Colony Forming Unit (CFU/ml) in GIT.

No.	Farms	Location	Chicken	Overall Average of CFU/ml in GIT	Log_{10} (CFU/ml)
1.	A	Kuala Pilah, N. Sembilan	Local	4.2043	0.6237
2.	B	Kuala Pilah, N.Sembilan	Local	0.9755	0.0107
3.	C	Meru, Klang, Selangor	Broiler	1.1240	0.0507
4.	D	Kapar, Klang, Selangor	Local	4.2652	0.6299
5.	E	Kuang, Rawang, Selangor	Broiler	4.1200	0.6148

The selected of coloring colonies were streaked again onto chromogenic agar plates (Figure 3) to get a pure culture for further species identification (Table 3). After 3 times restreaked onto chromogenic agar plate, a few colonies did not growth and maybe that cultures are unculturable which cannot survive in vitro condition. A set of questionnaire survey was also asked the owner farmer related due to antibiotic used for chickens and other farm management.

Based on the results, three chicken farms have a similar value of Log_{10}CFU/ml and Farm B and Farm C have the lowest value. This finding very important as food safety has a risk to humans associated with poultry contaminated with pathogens that could contribute significantly to food-borne diseases in humans (Hald *et al.*, 2016; Rouger *et al.*, 2017; Edgar, 2019) specifically when animals reared for their meat are colonized by bacterial pathogens, these pathogens can be spread to humans via the food chain (Tahiru *et al*., 2019).

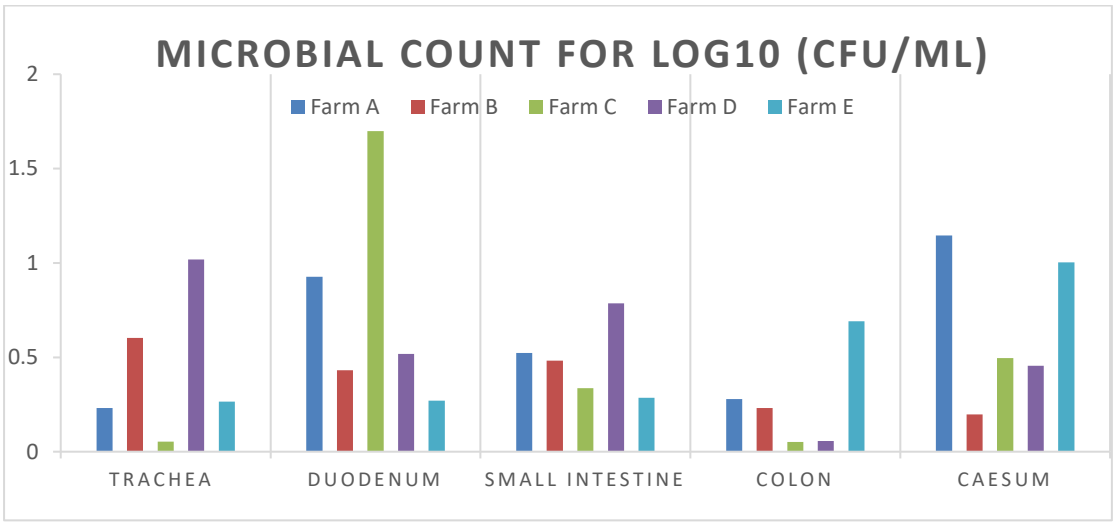

Figure 1: Microbial Count Colony Forming Unit (CFU)/ml in five parts of GIT from chicken farms.

Therefore it is significant interest in the selective sensing of anions by chromogenic and flurogenic methods in medical diagnostics, environmental, industrial monitoring and nuclear waste cleanup (Antonisse and Reinhoudt, 1998; Snowden and Anslyn, 1999; Beer and Gale, 2001; Thomas, Jolyand, and Swager, 2007). In the clinical microbiology laboratory, it is important, an accurate and timely fashion is challenging in diagnosis for patient care and infection prevention. Thus, clinical laboratories must assess their ability to identify bacteria fast and accurate. Therefore, Hata et al, 2020 reported that their teams have the ability to identify the *C auris* using chromogenic agar which spreading in health care facilities and cause outbreaks, rapid communication of suspected cases with institutional infection prevention providers and local, state, and national surveillance networks is essential to enhance awareness of this newly emerging yeast species.

4. Conclusion

ChromAgar Orientation medium was a good and fast discrimination presumptive of common species of pathogen bacteria from the mixed culture from GIT samples using direct plating. However, this finding in this study will further confirmation identification of species by biochemical and DNA sequencing.

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6. References

Antonisse, M.M.G., Reinhoudt, D.N., 1998. Neutral anion receptors: design and application, Chem. Commun. 443–448.

Beer, P.D., Gale, P.A., 2001. Anion recognition and sensing, Angew. Chem. Int. Ed. 40, 486–516.

Edgar, O Oviedo-Rondon. 2019. Holistic view of intestinal health in poultry. Journal of Animal Feed Science and Technology, Vol: 250: pg. 1-8

Hald, T., Aspinall, W., Devleesschauwer, B. 2016. World Health Organization estimates of the relative contributions of food to the burden of disease due to selected foodborne hazards: A structured expert elicitation. PLoS ONE, Vol. 11 (1). Article ID e0145839.

Hata, D. Jane, Romney Humphries, Shawn R. Lockhart. 2020. A Review: Candida auris An Emerging Yeast Pathogen Posing Distinct Challenges for Laboratory Diagnostics, Treatment, and Infection Prevention, Arch Pathol Lab Med—Vol 144.

Rouger, A., Tresse, O., and Zagorec, M. 2017. Bacterial contaminants of poultry meat: sources, species, and dynamics. Journal of Microorganisms. Vol. 5 (3). Article 50.

Snowden, T.S., Anslyn, E.V., Anion recognition: synthetic receptors for anions and their application in sensors, Curr. Opin. Chem. Biol. 3 (1999) 740–746.

Tahiru Mahami , Wellington Togby-Tetteh, Delali Isaac Kottoh, Leticia Amoakoah-Twum, Emmanuel Gasu, Sylvester Nana Yao Annan, Daniel Larbi, Isaac Adjei, and Abraham Adu-Gyamfi. 2019. Microbial Food Safety Risk to Humans Associated with Poultry Feed: The Role of Irradiation. Hindawi International Journal of Food Science. Article ID 6915736, 7 pages https://doi.org/10.1155/2019/6915736

Thomas, S.W., Jolyand, G.D., Swager, T.M., 2007. Chemical sensors based on amplifying fluorescent conjugated polymers, Chem. Rev. 107, 1339–1386.