EXPLORATION OF BACTERIAL CONTAMINATION IN SWIFTLET *(AERODRAMUS FUCIPHAGUS)* FAECES AT BONE REGENCY, SOUTH SULAWESI

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ABSTRACT

This study aimed to determine the presence of bacterial contamination found in faeces and determine the types of bacteria found in the swiftlet faeces. The research method used was the bacterial culture method using blood agar and Mac Conkey Agar so that it is then identified using the Vitek 2 compact tool. In this study the sample used was fresh faeces of swiftlet taken from wallet bird houses in five different regions in Bone Regency. Bacterial identification was carried out at the Public Health Laboratory Makassar, Indonesia. The sample weight used in each method was ± 5 grams. Identification of bacteria using Vitek 2 compact was by first culturing the sample in blood agar and Mac Conkey, after that the colony is taken and put into a tube containing 3 ml of suspension and then homogenized. Then the turbidity test was carried out using Densicheck until it reached 0.5 Mcfarland and put into Vitek 2 compact. The results of identification using the Vitek 2 compact system found that samples from rural areas contained 2 bacteria, *i.e.*, *Proteus mirabilis* and *Bacillus circulans*, samples from urban areas contained 2 bacteria *Escherichia coli* and *Klibsiella pneumonia,* from the mountain region found *Eschericia coli*, sample from the sea area found *Escherichia fergusoni*, and from rice fields found *Enterobacter aerogenes.*

Key words: Bacteria identification, swiftlet edible-nest, Vitek 2 compact, Bone Regency

Introduction

Swiftlet (*Aerodramus fuciphagus*) is one type of bird that produces nest products. The swiftlet builds nest from thick salivary secretions by the male swallow salivary glands. These nests function as breeding grounds, lay eggs and care for birds until they can fly (Guo *et al.,* 2006). In Asia the edible bird nest (EBN) has traditionally been widely used to maintain health (Hamzah *et al.,* 2013). Swiftlet's nest is used for hundreds of years as a food supplement in traditional Chinese medicine to overcome malnutrition, boost the immune system and increase the body's metabolism. Swallow nests contain high glycoprotein (Norhayati *et al.,* 2010). It is known that swiftlet nests have antioxidant, anti-inflammatory and bone-strengthening activities (Chua *et al.,* 2013). Nowadays, besides swiftlet nests which are considered very useful, swallow droppings are also very useful. Swiftlet manure is used as fertilizer because it is 'slow release' or slowly releases nutrients, so the availability of these nutrients can synergize with the age and growth of plants (Hariyadi, 2015). Bone Regency, is one of the districts that has a high swallow bird house population (Wijaya, 2017). Bone Regency is located on the East coast of South Sulawesi Province and is about 174 km from the city of Makassar. Bone Regency has a 138 km coastline. The eastern part of Bone Regency has a coastline along 138 km from south to north. The western and southern parts are mountains and hills with crevices in rivers (Pemda Kabupaten bone, 2013).

Microbial contamination is not only found in faeces but also in swallow's nests. It can occur when the nest is still in its habitat, when harvested, cleaned, washed, weighed, packed, marketed and until the bird's nest is ready for export. Swallow nests can be contaminated by bacteria wherein the bacteria are *Bacillus* sp., *E. coli*, and *Salmonella* sp. (Wong *et al.,* 2018). Based on the research results of Noerhayati (2010), it states that there are more than 480 bacteria that can be identified in the faeces of swallows in swallow bird houses in Sarawak Malaysia where 96% are Gram-positive bacteria and only 4% are Gram-negative bacteria, including *Staphylococccus* sp., *Bacillus* sp., *Lysinibacillus* sp., *Sporosarcina* sp., *E. coli*, *Paenibacillus* sp., *P. aeruginosa*, *L. airius*, and *Dermacoccus* sp. However, bacteria that are commonly used as indicators of faecal pollution is *Escherichia coli* so that direct physical contact with swallow faeces can cause concern from biological aspects and the level of environmental hygiene (Purnawijayanti, 2001). Bacterial contamination of food indicates the risk of various food-borne diseases that are harmful to public health (Susanna *et al*., 2010). Therefore, isolation and identification of bacteria were carried out in swallow bird houses in Bone Regency to determine the level of bacterial contamination in swallow feces in the Bone Regency area.

Materials and Methods

Collection of Swiftlet faeces

The samples of swiftlet feces being used in this research came from five different locations in Bone regency, South Sulawesi. Those locations are seashore, rice field, urban, rural as well as mountainous area. In each location, one sample was taken from a swiftlet's nest. Samples were taken from

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sterile plastic sheets placed in each corner of the swiftlet's nest. Samples were collected directly under the highest density swiftlet birds. Swiftlet droppings were taken in the afternoon to got excrement excreted when the swiftlet returns after foraging in the morning and afternoon. Swiftlet droppings on sterile plastic sheets were taken using sterile tweezers and put into a sterile tube (Saputra *et al*., 2018) then the collected impurities are brought into the laboratory to be inoculated into Brain-Heart Infusion Broth (BHIB) media as a fertilizing media

Bacterial Enrichment in Brain-Heart Infusion Broth (BHIB) Media

Swallow faeces samples as much as ±1 gram, then dissolved into 9 ml of BHIB fertilizer solution, incubated at 37ºC for 24 hours. Faeces that have been fertilized on BHIB media are inoculated on Blood agar and MacConkey then incubated at 37°C for 24 hours in an incubator, the next day it was observed growing colonies (Steven *et al.,* 2004).

Bacterial Culture in General Blood Agar (BA) Media

Bacteria that grew on BHIB media were then inoculated on Blood Agar (BA) media and incubated at 37°C for 24 hours. Blood Agar is a media that is enriched with additional nutrients that are rich for microbes; therefore BA media includes enriched and selective differential growth media because it supports the growth of various organisms and can give specific characteristics to certain groups of bacteria. Blood Agar media contains a mixture of tryptic soy agar and sheep blood, allowing bacterial differentiation based on their ability to lyse red blood cells. There are three types of blood hemolysis in BA media, namely beta hemolysis (β), alpha hemolysis (α) and gamma hemolysis (γ).

Bacteria Culture on MacConkey Agar Selective Media

Bacteria that grew on BHIB media were then inoculated on MacConkey media and incubated at 37°C for 24 hours (Wuryanti *et al.,* 2010). MacConkey Agar is a selective and differential media used to isolate Gram-negative enteric bacteria. MacConkey Media Agar contains lactose and bile salts. Bile salts can inhibit the growth of Gram-positive bacteria. Mac Conkey was a differential media because lactose acts as the primary carbon source, causing the growing colonies to be bacteria that can ferment lactose, such as *Escherichia coli* bacteria (Wahyuni *et al.,* 2018).

Gram Staining

Colonies that had grown on BA and MCA media were inoculated onto the slide. One drop of distilled water or 0.9% NaCl were taken on glass slide then the bacterial colony on the slide was fixed on a bunsen. The fixated preparations were dropped with crystal violet and then left for 1-2 minutes. The remaining dye was removed and then slide was rinsed with ethanol. Then the preparation was dropped with Lugol solution and leave for 30 seconds. The Lugol solution was discarded and rinse with running water. After that the preparation was dissolved using 96% alcohol until all the dyes wear off, and wash immediately with running water. Then after safranin dye was dropped and let stand for 2 minutes, then rinsed with running water and dried. Observation was done under a microscope with 100× objective magnification using emersion. Gram staining results showed that Gram-positive bacteria will turn purple while Gram-negative bacteria are red (Suarjana *et al.,* 2017).

Identification of bacteria using the Vitek 2 compact system

Colonies that had grown on Blood agar media and Mc Conkey media were taken and put into a tube containing 3 ml of suspension solution then homogenized. Then the turbidity test was done using Densicheck until it reached 0.5 Mcfarland. Then arrange the tubes on the cassette, place the Vitek 2 compact system card, in the order of identification. Vitek 2 compact system is a new generation microbiological analysis tool from Biomerieux which is based on the fluorescent method. This method can identify banter in a wide range including Gram negative (GN), Gram Positive (GP), anaerobic and Corynebacterium (ANC), Bacillus (BCL), Neisseria haemophilus (NH), and fungus (YST).

Results and Discussions

Based on research results, from 5 fresh swiftlet faeces samples obtained as many as 1 Gram positive bacterial isolates and 4 Gram negative bacterial isolates. And from these bacteria all are categorized as pathogens to humans. Samples from rural areas obtained 2 bacterial isolates namely *Proteus mirabilis* and *Bacillus circulans*. Samples from the urban area were identified as *Escherichia coli* and *Klebsiella pneumonia*. Samples from the mountain region found 1 bacterial isolate *Eschericia coli*, from the sea area found 1 bacterial isolate *Escherichia fergusoni,* and sample from paddy fields found 1 bacteria *Enterobacter aerogenes*.

Bacteria can be isolated from 5 samples obtained. Table 1 presented the macroscopic characteristics including the color, shape, size, and surface of growing bacteria.

The growth of bacteria in blood agar or blood can help in identifying bacteria by looking at the morphology of bacterial colonies which include size, shape, surface, elevation, color and hemolysis (Murwani 2015). Based on observations of colony morphology (Table 1), the results shows that all colonies have sizes ranging from small to large. Bacteria have different

Table 1: Macroscopic observations of BA media bacterial isolates from swiftlet feces samples in Bone Regency

	Sampling Location						
Morphology	1		2	3		4	5
Shape	bk1	bk2	bk1	bk1	bk1	bk2	bk1
Colour	wk1	wk4	wk2	wk1	wk2	wk1	wk2
ColonySurface	pk2	pk2	pk2	pk2	pk2	pk1	pk1
Colony Size	uk2	uk2	uk2	uk2	uk3	uk1	uk1
Hemolisis	-	-	-	-	-	+	-

Note: Sampling locations: (1) rural area samples (2) urban area samples (3) mountainous area samples (4) sea area samples (5) rice field samples. Colony shape: (bk1) round (bk2) is irregular. Colony color: (wk1) white (wk2) clear (wk3) red (wk4) green. Colony surface: (pk1) flat (pk2) convex. Colony size: (uk1) small (uk2) medium (uk3) large. Hemolysis of the colony: (-) does not hemolysis (+) hemolysis

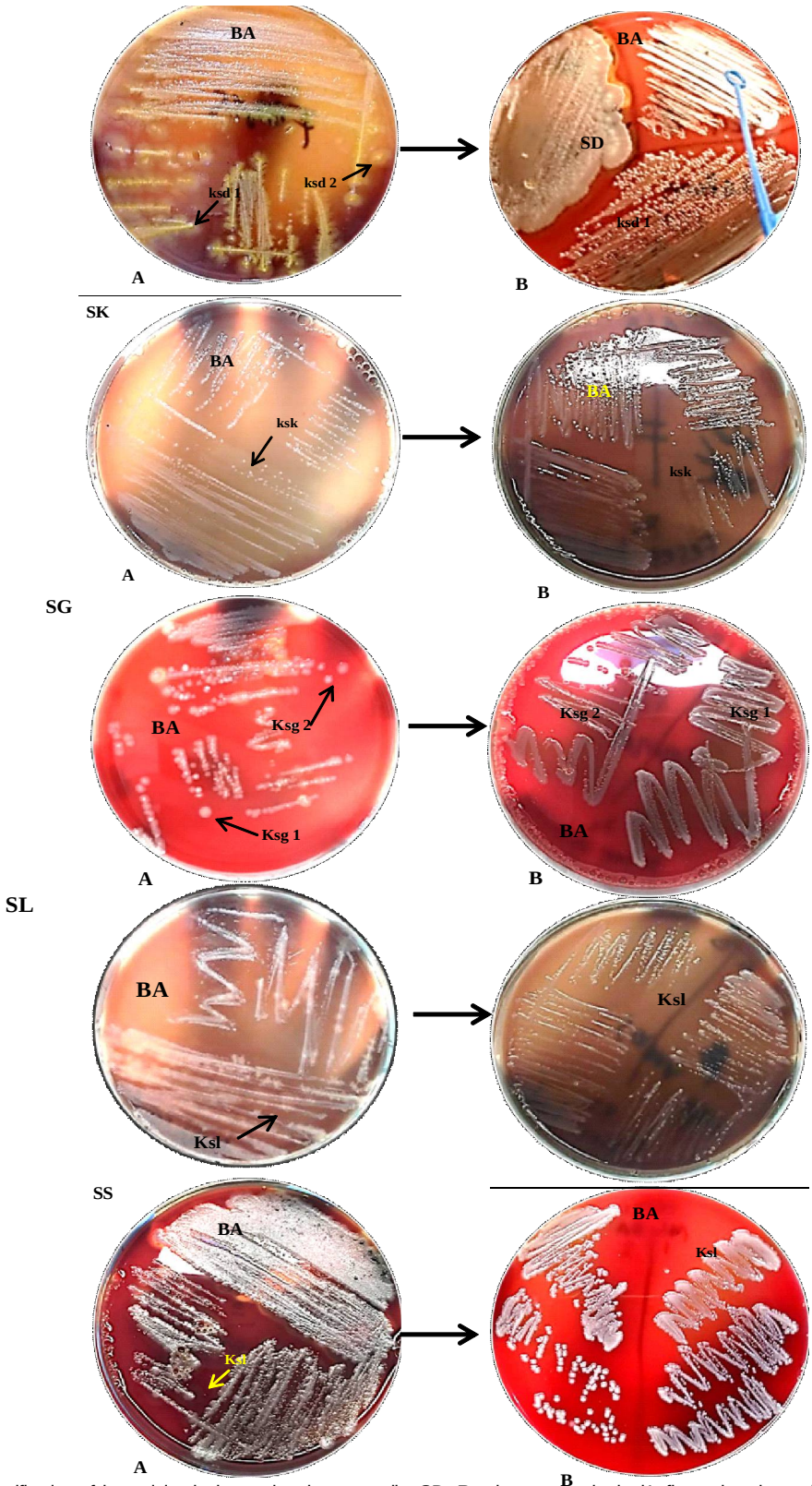


Fig. 1: Reading and purification of bacterial colonies on bood agar media. SD: Rural area sample, ksd1: first colony in rural sample (round, white, convex, medium), ksd2: second colony in rural sample (irregular, green, convex, and medium), A: overall colony reading, B : purified colony, SK: sample of city area, ksk: colony of city sample (round, clear. Convex, medium), SG: mountain area sample, ksg1: first colony of mountain sample (round, white, convex, medium), ksg2: second colony of mountainous samples (round, clear, convex, large). SL: sea area sample, ksl: colony of sea sample (irregular, white, flat, small. SS: rice field sample, kss: colony of rice sample (irregular, clear, convex, large) BA: bood agar (+): can hemolysis of the blood, (-): unable to haemolysis of the blood

Table 2. Results of macroscopic observations of MC media bacterial isolates from swiftlet faeces samples in Bone Regency

Morfologi	Sampling Location										
	1			2		3		4			5
Shape	bk	bk	bk	bk1	bk	bk1	bk1	bk	bk	bk	bk2
Colour	wk	wk	wk	wk3	wk	wk1	wk3	wk	wk	wk	wk1
ColonySurface	pk	pk	pk	pk2	pk	pk1	pk1	pk	pk	pk	pk2
Colony Size	uk	uk	uk	uk2	uk	uk2	uk3	uk	uk	uk	uk3

Note: Sampling locations: (1) rural area samples (2) urban area samples (3) mountainous area samples (4) sea area samples (5) rice field samples. Colony shape: (bk1) round (bk2) is irregular. Colony color: (wk1) white (wk2) clear (wk3) red (wk4) green. Colony surface: (pk1) flat (pk2) convex. Colony size: (uk1) small (uk2) medium (uk3) large.

haemolysis abilities. Sample 4 had the ability to hemolyse blood agar, while isolates 1, 2, 3 and 5 did not hemolysis blood agar. Can be seen in Figure 1.

Based on observations of colony morphology (Table 2), the results show that all colonies have sizes ranging from small to large. From these observations, a purification was made to obtain a pure colony from each colony so that it can then be tested on the Vitek colony. As explained in the Figure 2.

The results of observations using the Vitek 2 compact system analysis results include:

McFarland: (0.50 - 0.63)

Identification Information	Card:	GN	Lot Number:	2410762113	Expires: Dec 28, 2019 12:00 SGT
	Completed:	Oct 23, 2019 17:59 SGT	Status:	Final	Analysis Time: 3.83 hours
Organism Origin	VITEK 2				
Selected Organism	99% Probability	Escherichia coli			
	Bionumber:	0405610450426610			Confidence: Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

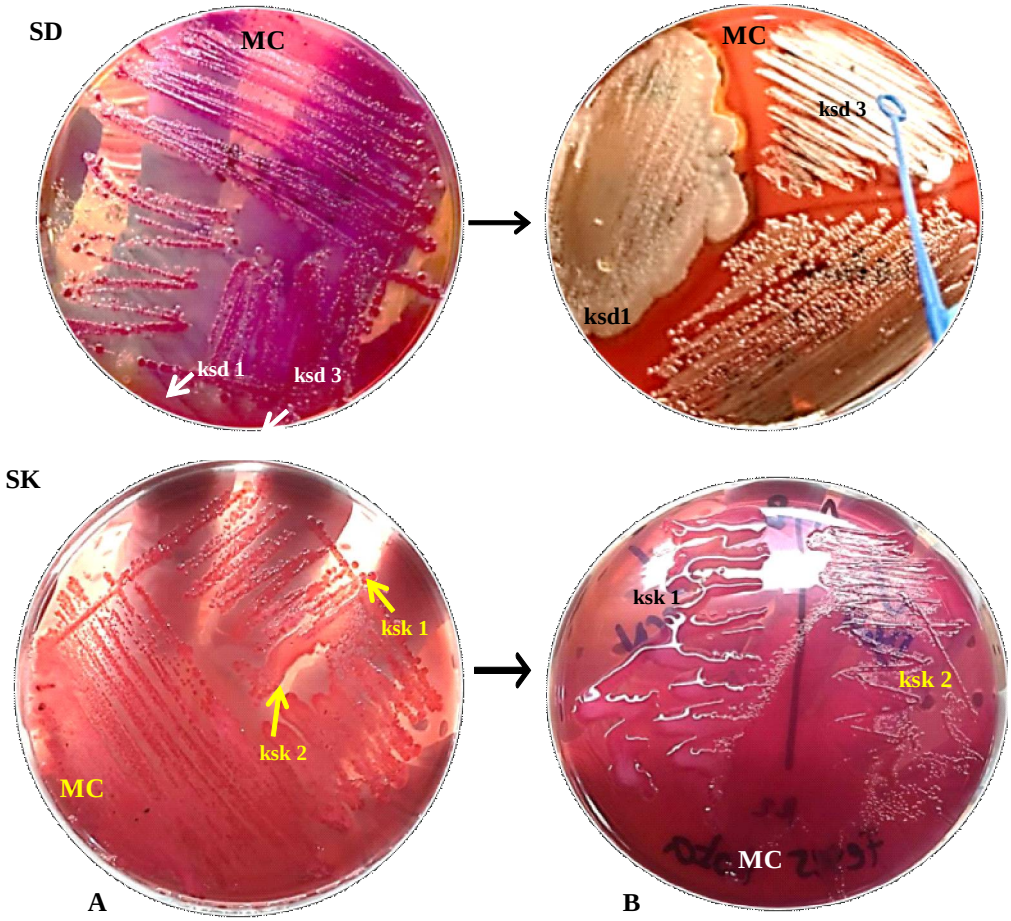
Fig. 3. Results of identification of *Proteus mirabilis* bacteria using Vitek 2 compact system

Proteus mirabilis

Bacteria Mirabilis is found in swiftlet faeces samples from rural areas. The results of identification using the Vitek 2 compact system, showed that 95% of the bacteria were *Proteus mirabilis*, can be seen in Figure 3.

Bacillus circulans

Bacillus circulans is found in swallow faeces samples



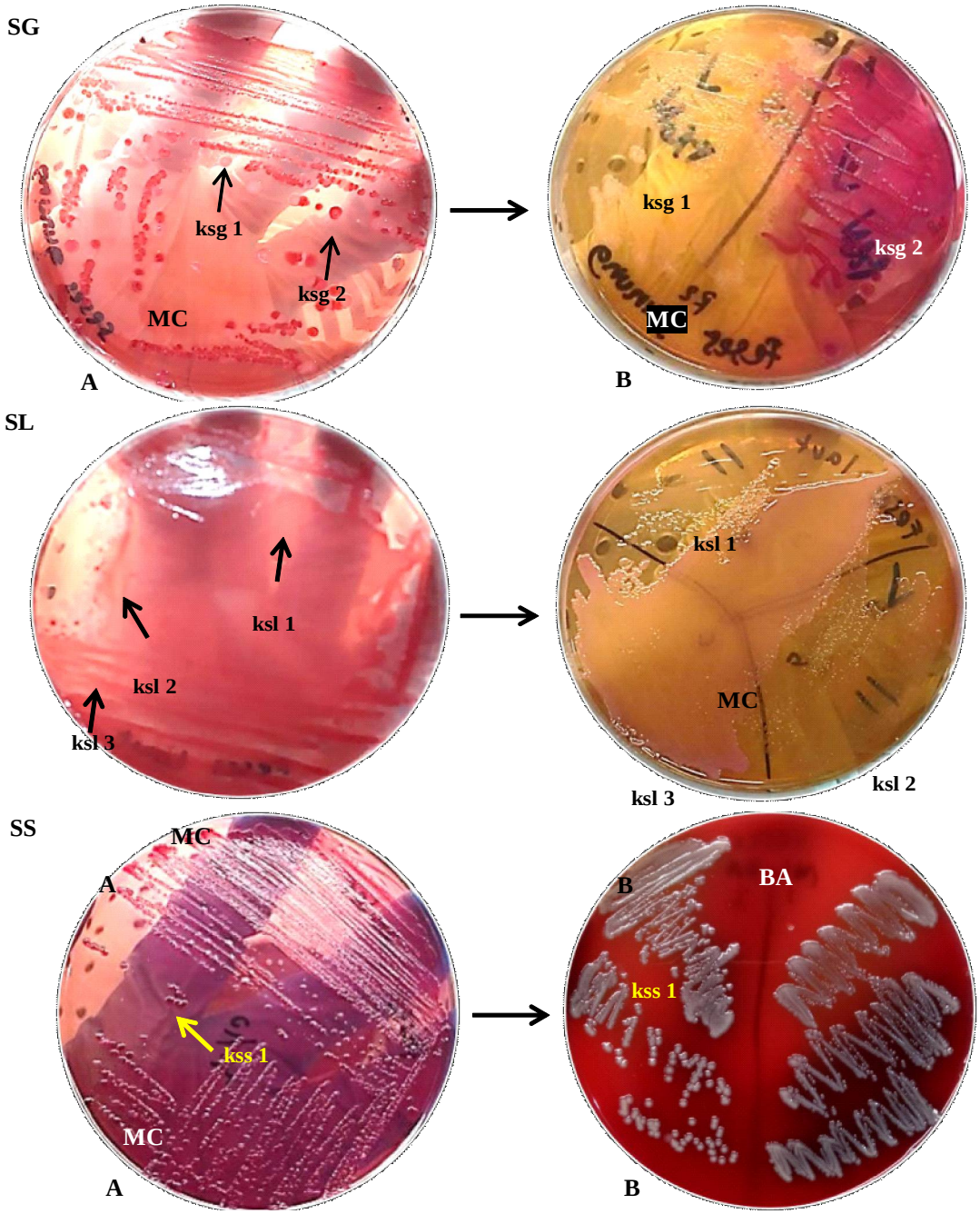


Fig. 2: Reading and purification of bacterial colonies on MacConkey media. SD: Rural area sample, ksd1: first colony in rural sample (round, red, convex, and small), ksd2: second colony in rural sample (round, red, convex, and medium), ksd3: third colony of sample rural (irregular, white, flat, large). A: overall colony reading, B: refined colony, SK: city area sample, ksk1: first colony of city sample (round, red. Convex, medium), ksk2: second colony of city sample (round, white, convex , is). SG: mountainous region sample, ksg1: first colony of mountain sample (round, white, flat, medium), ksg2: second colony of mountain sample (round, red, flat, large). SL: sea area sample, ksl1: first colony of sea sample (round, white, convex, small), ksl2: second colony of sea sample (round, clear, convex, small), ksl3: third colony of sea sample (round, round, red, convex, medium), SS: rice field sample, ks1: first colony of rice sample (irregular, white, convex, large). MC: MacConkey media, BA: Blood Agar media

from rural areas. The results of identification using the Vitek 2 compact system using the BCL card is a card that is used specifically to identify Bacillus sp. Bacteria, showing that 95% of *Bacillus circulans* bacteria can be seen in Figure 4.

Klebsiella pneumonia

Klebsiella pneumonia is found in swiftlet faeces samples from urban areas. The results of identification using the Vitek 2 compact system, showed that 88% had the bacteria *Klebsiella pneumonia*, can be seen in Figure 5.

Escherichia coli

Escherichia coli is found in swiftlet faeces samples from urban and mountainous areas. The results of identification

using the Vitek 2 compact system, showed that 98% were *Escherichia coli* bacteria in urban areas and 99% *Escherichia coli* bacteria were found in mountainous regions, can be seen in Figure 6.

Eschericia fergusonii

Eschericia fergusonii bacteria were found in swiftlet faeces

McFarland: (0.50 - 0.63)

Identification Information	Card:	GN	Lot Number:	2410762113	Expires: Dec 28, 2019 12:00 SGT
	Completed:	Oct 23, 2019 17:59 SGT	Status:	Final	Analysis Time: 3.83 hours
Organism Origin	VITEK 2				
Selected Organism	99% Probability	Escherichia coli			
	Bionumber: 0405610450426610				Confidence: Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

Fig. 4: Bacteria identification results *Bacillus circulans* using Vitek 2 compact system

McFarland: (0.50 - 0.63)

Identification Information	Card:	GN	Lot Number:	2410762113	Expires: Dec 28, 2019 12:00 SGT
	Completed:	Oct 23, 2019 17:59 SGT	Status:	Final	Analysis Time: 3.83 hours
Organism Origin	VITEK 2				
Selected Organism	99% Probability	Escherichia coli			
	Bionumber: 0405610450426610				Confidence: Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

Fig. 5: Bacteria identification results *Klebsiella pneumonia* using Vitek 2 compact system

McFarland: (0.50 - 0.63)

Identification Information	Card:	GN	Lot Number:	2410762113	Expires: Dec 28, 2019 12:00 SGT
	Completed:	Oct 23, 2019 17:59 SGT	Status:	Final	Analysis Time: 3.83 hours
Organism Origin	VITEK 2				
Selected Organism	99% Probability	Escherichia coli			
	Bionumber: 0405610450426610				Confidence: Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

Fig. 6: Bacteria identification results *Escherichia coli* using Vitek 2 compact system

samples from paddy fields. The results of identification using the Vitek 2 compact system showed that 99% of the bacteria are *Eschericia fergusonii* in the area close to the sea can be seen in Fig.7.

All bacteria grown from 5 samples in 5 different regions in Bone District produced positive results using 2 compact Vitek devices. The bacteria that were identified were *Proteus*

McFarland: (0.50 - 0.63)

Identification Information	Card:	GN	Lot Number:	2410762113	Expires: Dec 28, 2019 12:00 SGT
	Completed:	Oct 23, 2019 17:59 SGT	Status:	Final	Analysis Time: 3.83 hours
Organism Origin	VITEK 2				
Selected Organism	99% Probability	Escherichia coli			
	Bionumber: 0405610450426610				Confidence: Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

Fig. 7: Bacteria identification results *Eschericia fergusonii* using Vitek 2 compact system

mirabilis, *Bacillus circulans*, *Klebsiella pneumonia*, *Escherichia coli* and *Eschericia fergusonii*. The bacteria identified mostly showed the similarity of bacterial findings in the study of Leong *et al.* (2013) namely *Escherichia coli*, *Proteus* sp., and *Bacillus* sp. However, in the study of Leong *et al.* (2013) more bacteria were found because they chose from 10 different locations.

The media used in this study were blood agar and MacConkey. Blood agar is a solid and differential media. Differential media are media that are added with certain chemicals so that a microorganism forms a growth to classify a group of types of bacteria. Blood agar distinguished between hemolytic and nonhemolytic bacteria based on their ability to lyse red blood cells.

There are three types of hemolysis, namely beta hemolysis, alpha hemolysis and gamma hemolysis. Beta hemolysis is a complete lysis of red blood cells and hemoglobin, alpha hemolysis refers to partial lysis of red blood cells and hemoglobin. This results in a change of color around to greenish-gray. In gamma hemolysis there is no hemolysis where there is no change in color in the media.

Proteus mirabilis is gram negative, it is in the form of a short stem, non spherical, generally moves with flagella, peritricus, the colonies spread on agar media. *Proteus mirabilis* does not ferment lactose but ferments glucose in the presence of gas (Manos *et al.,* 2006). *Proteus mirabilis* is a significant pathogen in the urinary tract. Urinary tract infections begin with bladder colonization, which causes cystitis. Then, the infection continues to the kidneys, which leads to acute pyelonephritis, chronic inflammation and eventually kidney failure, which if left untreated, can cause death.

Bacillus circulans are gram-positive, have spores, ellipsoidal spores are subterminal or terminal, located at the center and cylindrical in shape like the kidney organ shape. These bacteria are facultative anaerobes and can multiply in the blood and host tissues without an effective immune response. *Bacillus circulans* is one of the agents that cause bacteremia and sepsis in patients of large hospitals. Sepsis is a systemic inflammatory response to an infection that causes damage to the body's vital organs such as the heart, lungs,

kidneys and liver.

Klebsiella pneumoniae is a gram-negative (-) bacterium, in the form of a short rod, having a size of 0.5-0.5 × 1.2 µ. This bacterium has capsules, but does not form spores. *K. pneumoniae* is unable to move because it has no flagellum but is able to ferment carbohydrates to form acids and gases. Based on their need for oxygen, *Klebsiella pneumoniae* is an anaerobic facultative bacterium. *Klebsiella pneumonia* can ferment lactose. *Klebsiella pneumoniae* species show mucoid growth, large and non-motile polysaccharide capsules (Anderson *et al*., 2007). In humans *Klebsiella pneumoniae* can cause pneumonia, which attacks the lung tissue (alveoli). *Klebsiella pneumoniae* causes lung disease in the form of swelling of the lungs so that the left and right lobes of the lungs become unequal, fever (chills), coughing (bronchitis), thickening of the mucosal walls and sputum bleeding. In addition, this bacterium can also cause urinary tract infections, and nosocomial infections (Beesley, 1983).

Escherichia coli are gram-negative bacillus from the family Enterobacteriaceae. These bacteria are facultative anaerobes, can cause infections outside the intestines such as cystitis, cholecystitis, appendicitis, peritonitis, pyelonephritis, infection of postoperative wounds, meningitis and sepsis. Bacterial infections often also in the urinary tract with signs and symptoms that are not typical of *Escherichia coli* infection. It also can infect the digestive tract and can cause diarrhea with different mechanisms (Jawed *et al*., 2005).

Escherichia fergusonii **i**s a gram-negative, facultative, rod-shaped anaerobe, which does not form spores that are commonly owned by the Enterobacteriaceae family. *Escherichia fergusonii* was recently known as an emerging bacterial pathogen. Previously known as Enteric Groups 10 and 19, this bacterium is a new species in the family Enterobacteriaceae. *Escherichia fergusonii*, can be isolated from clinical specimens, from the intestines of humans and warm-blooded animals (Mahapatra, 2005).

Conclusions

All bacteria grown from 5 samples in 5 different regions in Bone Regency produced positive results using 2 compact Vitek devices. The bacteria that were identified were *Proteus mirabilis*, *Bacillus circulans*, *Klebsiella pneumonia*, *Escherichia coli* and *Eschericia fergusonii.*

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Author contribution

AMSA and BY contributed to the main design of this research, supported the experiments, wrote, reviewed and edited the manuscript. NUR do the experiments, collected and analyzed the data. AWJ, DKS and WSM reviewed and edited the manuscript. All authors approved the final version of the manuscript and agreed to publish it.

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