ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM THE SECRETION OF CHRONIC SUPPURATIVE OTITIS MEDIA

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ABSTRACT

The aims of this research were to isolate and identify the pathogenic bacteria in the secretion of Chronic Suppurative Otitis Media (CSOM) patients as the development of Lactic Acid Bacteria (LAB) analysis in Virgin Coconut Oil (VCO) fermentation process. We hope LAB in the VCO could be antimicrobial/antibacterial of bacteria in the secretion of CSOM patients. Suryani (2014) said that the isolate of LAB could be antibacterial against 5 samples of bacteria (*E.coli NBRC14237, Staphylococcus aereus NBRC 13276, Bacillus substilis BTCCB, Salmonella thypii,* and *Listeria monocytogenes)* and also against 2 samples of fungi (*Aspergillus niger* and *Candida sp.)*. Chavan et al.(2014) and Sharma (2014) said bacteria that cause CSOM were *Pseudomonas aeruginosa, Klebsiella, Proteus, Staphylococcus aureus* and fungi called *Aspergillus spp* and *Candida spp.* This research was conducted in 2 stages; (1) isolation of the bacteria in the secretion of CSOM patients using blood agar and dilution method; (2) identify the isolates morphologically, physiology, and other biochemical test. We got 126 isolates and 5 kinds of pathogenic bacteria (*Pseudomonas aureginosa, Staphilococus aureus, Staphilococus epidermidis, Proteus mirabilis, Klebsiella Sp)* and one kind of fungi (*Candida sp)*. The samples of CSOM patients are 60% above aged 20 and 40% under aged 20, 50% of them are male, and 50% of them are female.

Keywords: Pathogenic bacteria isolation, Secretion of CSOM patients, Chronic suppurative otitis media, Virgin Coconut Oil (VCO), Lactic Acid Bacteria (LAB)

INTRODUCTION

Chronic Suppurative Otitis Media (CSOM) is a kind of ear disease that usually attacks children and causes deafness, even dead (Lee, 2009). It usually attacks people in developing countries such as India, Nepal, Vietnam and also Indonesia (Rajahmundry, 2014). Indonesian calls it '*congek'*, and it is a deadly disease because there is tympanic membrane perforation and secretion that flows from the outer ear continuously or temporary and it can cause dangerous complication such as brain abscess and meningitis (Djafaar *et al.*, 2007). CSOM happens because the late effect of treatment for acute otitis media patient, or poor hygiene practice, high virulence, and weak immune system because of malnutrition (Djafaar *et al.*, 2007).

There were many researchers have tried to isolate the pathogenic bacteria in the secretion of CSOM patients. One of them was an Indian researcher, Prakash M (2013) that said, from 80 samples of CSOM patients, there were few pathogenic bacteria; *Staphilococcus aureus, Pseudomonas sp, Escherichia coli,* and *Klebsiella sp., a*pparently 18% of the bacteria were resistance toward antibiotic like methicillin, and sensitive toward amikacin, chloramfenicol and piperacillin.

Helen M (2009) reported that the most pathogenic bacteria found in CSOM object were *Streptococcus Pnemonea* and a virus. Edwar, Yan et al (2015) reported that pathogenic bacteria in the secretion of CSOM patients were aerobic and anaerobic and most of them were *P.aeruginosa*, *S.aureus*, *S. pyogenes*, *K.pneumoniae*, *H.influenzae*, *Bacteroides* and *Proteus sp.* The mixture of aerobic and anaerobic bacteria that form a layer called biofilm.

Meanwhile, Suryani et al (2014) and Luz Andriana Sarmiento (2010) said that there were bacteriocins in Lactic Acid Bacteria (LAB). Bacteriocins can kill pathogenic bacteria but it is not dangerous for non-pathogenic bacteria (Nguyen, 2010). Suryani *et al.* (2016) also said that after doing antibacterial test and anti-function test using 5 samples of bacteria (*E.coli NBRC14237, Staphylococcus aereus NBRC 13276, Bacillus substilis BTCCB, Salmonella thypii,* and *Listeria monocytogenes)* and 2 samples of fungi (*Aspergillus niger* and *Candida sp)* in VCO fermentation process, there were pathogenic bacteria of CSOM patients found among samples of bacteria (*S. Aureus*) and there was a fungus of CSOM patient between the samples.

Because oil layer in VCO contains LAB that can inhibit the growth of pathogenic bacteria, we hope that pathogenic bacteria in secretion of CSOM patients can be inhibited also by the LAB.

RESEARCH METHOD

This research was conducted from April until October 2016. The secretions were taken from CSOM patients in X Hospital. Data analyzes was conducted in Central Laboratory of X Hospital, Basic Laboratory of Kopertis X, and Microbiology Laboratory of Muhammadiyah University of West Sumatera.

Materials

Material in this research was ear liquid of 126 CSOM patients in X Hospital. The media to grow the bacteria during conventional isolation and identification processes were blood agar and McConkey agar. MRS (15g peptone, 5g yeast extract, 10g dextrose, 5g tomato juice, 2g monopotassium phosphate, and 1g polysorbate 80), Luria-Bertani medium (10g tryptone, 5g yeast extract, and 10g NaCl), sodium acetate, liquid nitrogen, methylene blue, sterile aquadest, sodium azide, HCl 6 N, ampicilin, ammonium sulfate, Tris-HCl 50 mM pH 7.4, NaCl 1 M, Tris-HCl 100nM pH 8.5, glycerol, isopropanol, 70% ethanol, ammonium molybdate, trisodium citrate, aquabidest, methanol, pure Agar, 70% alcohol, 96% ammonium sulfate (NH4)2SO4, Aquadest, buffer solution pH 7, technical hydrogen peroxide (H2O2), potassium hydroxide (KOH), phenolphthalein (PP) analysis, technical starch, and lactose broth.

Method

There were 2 stages in this research; (1) isolation of pathogenic bacteria of 125 CSOM patients; (2) Identification of pathogenic bacteria using gram-negative and positive test, bacterial staining test, morphology test, and biochemical test such as catalase test and other carbohydrate tests.

The Isolation of Pathogenic Bacteria in the Secretion of the Patients

We do isolation stage before doing the identification of pathogenic bacteria in the secretion of 126 CSOM patients. Pathogenic bacteria from 126 CSOM patients are isolated using dilution method until 10^{-7} dilution level and the media used to isolate the pathogenic bacteria are

blood agar and McConkey agar. Streak the bacteria for a single colony and it will be the isolate of the pathogenic bacteria. Along with the secretion in blood agar, the secretion is also enriched in tiogikolat. If there is no bacterium that grows in the media, we can take the sample that has been already enriched and growth in blood agar. Every CSOM patient usually can produce one isolate.

The Identification of Isolate of Pathogenic Bacteria

Isolates that have been collected are identified morphologically by seeing the colony pattern, and the color of the colony. Positive and negative-gram test, biochemical test such as catalase test, starch test, and novobiocin test are also conducted.

RESULT AND DISCUSSION

Data of CSOM patients in this research can be seen in Table 1 below:

Tabel 1. Sample Distribution

No.	Pasien	Jumlah	%
1.	Anak-anak (dibawah 13 tahun)	51	40
2.	Dewasa	75	60
3.	Laki-laki	72	57
4.	Perempuan	54	43

From table above we also can see that most of them are men (57%). And we also can see that 40% of the CSOM patients in this research are children, and 60% are adult. Yaor, MA (2006) in his previous research said that CSOM can attack children and adult. 73 CSOM patients in his research aged 9-84, 17% of them were children aged 9-15. It happened because of poor hygiene practice. Meanwhile, Shyamala (2012) found that 70% of CSOM patients were children aged 0-20.

The Isolation of Pathogenic Bacteria

Usually we can find one kind of pathogenic bacteria of the CSOM patient in the isolation process, and in this research we got 126 isolates. AH Singh (2012) in his previous research found one isolate in 64% of 192 samples, 34% of them had more than one, and 5.33% of the isolated secretion produced fungi.

Morphologic Identification of Pathogenic Bacteria

The result of pathogenic bacteria identification of 126 secretions of CSOM patients can be seen in Table 2 below:

Tabel 2. Morphologic Analysis of the Isolates

No.	Macroscopic Characteristics of Isolate	Isolate	Number of Isolate
1.	- The color is grayish white - The shape like a fragment - The size is 6-15 mm - The texture is rough - Greenish pigment - Smelly - Gram-negative (bacilli)	*Pseudomonas aureginosa*	74 (58,7%)
2.	- Circular shape - The size is medium - Convex - Possessing flagella - Spread - Smell salty - Gram-negative (bacilli)	*Proteus mirabilis*	21 (16,6%)
3.	- Circular shape - The size is big - Convex - Mucoid - Shiny - The edge is smooth - Gram-negative bacilli	*Klebsiella*	7 (5%)
4.	- Circular shape - Slightly Convex - The edge is smooth - The color is yellowish white - The size is 2-5 mm - β hemolytic - Positive-gram (cocci) - Aciniform (Grouped like grapes)	*Staphylococcs aureus*	14 (11%)
5.	- Circular shape - Slightly Convex - The edge is smooth - The color is white - The size is small - Cocci - Positive-gram - Aciniform (Grouped like grapes)	*Staphylococcus epidermidis*	4 (3%)

Meanwhile in fungi identification, when we were doing observation of isolation process, we found a colony that had hypha in it. We did gram staining test and the result was positive pseudohyphae. Then, the samples were growth in blood agar and saboraud agar. The colony did not grow in blood agar but grew in saboraud agar in circular shape, white, and slightly mucoid. The result can be seen in Table 3 below:

Tabel 3. Morphologic Analysis of Fungi from the isolates of Pathogenic Bacteria

No.	Characteristics	Isolate	Number of Isolate
1.	- Positive-gram - Pseudohypha + - Didn't grow in blood agar - Grow in saboraud - Circular shape, white, and slightly mucoid	*Candida sp*	6 (4,7%)

Biochemical Test

The result of biochemical test of the isolates can be seen in Table 4 below:

Tabel 4. Result of Biochemical Test of the Isolate

No.	Test	Result	Number of Isolate	Isolate
1.	TSIA	K/K	74	*Pseudomonas aureginosa*
2.	Gas	+		
3.	H2S	_		
4.	SC	+		
5.	Sulfur	+		
6.	Indole	-		
7.	Motile	+		
1.	TSIA	K/A	21	*Proteus mirabilis*
2.	Gas	+		
3.	H2S	+		
4.	SC	+		
5.	Sulfur	+		
6.	Indole	-		
7.	Motile	+		
1.	TSIA	A/A		*Klebsiella*
2.	Gas	+		
3.	H2S	-		
4.	SC	+		
5.	Sulfur	-		
6.	Indole	-		

7.	Motile	-		
1.	Catalase	+	14	*Staphylococcus aureus*
2.	Gas	+		
3.	Coagulase	+		
4.	Novobiocin	Sensitive		
1.	Catalase	+	4	*Staphylococcus epidermidis*
2.	Gas	+		
3.	Coagulase	-		
4.	Novobiocin	Sensitive		

From the result of morphology identification in Table 2 and 3, we can see the shape, color, size of colony from each isolate and also the gram test result. From all result above, the pathogenic bacteria in the secretion of CSOM patients in X Hospital are *Pseudomonas aureginosa* (58,7%), *Staphilococus aureus* (11 %), *Staphilococus epidermidis* (3%), *Proteus mirabilis* (16,6 %), *Klebsiela sp* (5%) and 1 fungi *Candida sp* (4,7%). This result is supported by the result of biochemical test in table 4. This matter also has been reported by other scientists but there were a few differences about the pathogenic bacteria and fungi found in the secretion of CSOM patients. Sthrestha et.al (2011) said that pathogenic bacteria and pathogenic fungus of CSOM patients were *Staphylococcus aureus* 32,2%, *Streptococcus pnemoni* 6,1 %, *Pseudomonas aureginosa* 26,9 % , *Klebsiella sp* 10,4 %, *Proteus mirabilis* 6,9 %, *E.coli* 6,9%, fungi *Aspergillus sp* 6,9 % *Candida sp* 2,6 %.

CONCLUSION

From all result above, we can conclude that:

1. There were 126 isolates of pathogenic bacteria from the secretion of CSOM patients
2. There were 5 kinds of pathogenic bacteria in the secretion of CSOM patients in X Hospital; *Pseudomonas aeruginosa; Klebsiella, Proteus; Staphylococcus aureus; Staphilococus epidermidis* and one species of fungi *Candida spp.*

RECOMMENDATION

We recommend other researchers to identify the pathogenic bacteria and pathogenic fungi molecularly using PCR in order to get the more accurate identification result.

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REFERENCE

Gopala Kresna.2010. Coconut Oil: Chemistry,Production and Its Applications –A ReviewIndian Coconut Journal. July 2010 .page 15-27

Helen, M (2009), "Otitis media: viruses, bacteria, biofilms and vaccines", **MJA•**191 (9),S44-S49

Handayani Rini, Joko Sulystio, Rita Dwi Rahayu. 2009. Extraction of Coconut Oil (*Cocos nucifera* L.) through Fermentation System. BIO DIVERSITAS Volume 10, Number 3, ISSN: 2085-4722 (electronic)Pages: 151-157

Luz-Adriana Sarmiento-Rubiano1, Bernard Berger2, Déborah Moine2, Manuel Zúñiga1, Gaspar Pérez-Martínez1,María J Yebra1. 2010. Characterization of a novel *Lactobacillus* species closely related to *Lactobacillus johnsonii* using a combination of molecular and comparative genomics methods. Sarmiento-Rubiano et al. BMC Genomics, 11:504

Mustaque Ali Mushtaque Ali Memon, Salman Matiullah (2008). Frequency of Un-Safe Chronic Suppurative Otitis Media in Patients with Discharging Ear JLUMHS MAY - AUGUST 2008.

Nguyen H.T.H., F.B. Elegado, N.T. Librojo-Basilio, R.C. Mabesaand E.I. Dizon. 2010. Isolation and characterisation of selected lactic acid bacteria for improved processing of *Nemchua*, a traditional fermented meat from Vietnam. Beneficial Microbes; 1(1): 67-74.

Prakash M, Laksmi K, (2013), Bacteriological Profile and Ttheir Antibiotic Susceptibility Pattern of Cases of Chronic Suppurative Otitis Media. *Asian J Pharm Clin Res, Vol 6, Suppl 3, , 210-212*

Rajahmundry. 2012. Aerobic bacteriology of chronic suppurative otitis media in Rajahmundry, Andhra Pradesh, India. *Biology and Medicine, 4 (2): 73-79.*

Rajahmundry (2014), " Biofilm Pada Otitis Media Supuratif Kronis", JMJ, 3(1), Hal: 68– 78

Shyamala,R, (2012) ," The study of bacteriological agents of chronic suppurative otitismedia - Aerobic culture and evaluation", J. Microbiol. Biotech. Res., 2012, 2 (1):152-162

Sadiah. 2011. Virgin coconut oil production by fermentation using *Saccharomyces cerevisiae. Proceedings of the 2nd International Seminar on Chemistry 2011 (pp. 141-144)*

Sri Redjeki and Ely Kurniati. 2013. The Kinetic Reacion of Virgin Coconut Oil (VCO) Fermentation in an Ideal Bioreactor Tank in a Batch Process. J. Chem. Chem. Eng. 7 : 159-163.

Shyamala and P Sreenivasulu Reddy.(2012). The study of bacteriological agents of chronic suppurative otitismedia - Aerobic culture and evaluation J. Microbiol. Biotech. Res., , 2 (1):152-162

Singh,AH, (2012), "Aerobic bacteriology of chronic suppurative otitis media in Rajahmundry, Andhra Pradesh, India", *Research Article Biology and Medicine, 4 (2):* 73-79.

Suryani, Abdi Dharma.(2014). Antimicrobial and Antifungal Activity of Lactic Acid Bacteria Isolated from Coconut Milk Fermentation.Research Journal of Pharmaceutical, Biological and Chemical Sciences 5(6) page 1587-1595.

Suryani, et.al, (2016)," Isolation and Characterization of Bacteriocin latobacillus plantarum strain nm178-5 from fermentation process which contained on coconut milk",Transylvanian Review 24(6),p 614-628.

Suryani, Abdi Dharma. 2014. DDBJ (Data DNA Bank Jepang) (*accession number*) AB890143.