UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF POTENTIAL BACTERIAL PATHOGEN IN LABORATORY RATS PRODUCED LOCALLY

NUR ASYIKIN BINTI HARON

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IDENTIFICATION OF POTENTIAL BACTERIAL PATHOGEN IN LABORATORY RATS PRODUCED LOCALLY

NUR ASYIKIN BINTI HARON

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Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
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It is hereby certified that we have read this project paper entitled "Identification of potential bacterial pathogen in laboratory rats produced locally", by Nur Asyikin binti Haron and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year project.

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Assoc. Prof. Dr. Abdul Rahim Abdul Mutalib
DVM (Mymensingh), MS (UC Davis), PhD (Cambridge)
Lecturer,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

____

Assoc. Prof. Dr. Siti Khairani Bejo
DVM (UPM), MVSc (UPM), PhD (UPM)
Lecturer,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-supervisor)

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DEDICATIONS

To Ayah and Ibu,

To my family,

Especially my aunts and cousins,

For your endless love and support.

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ACKNOWLEDGEMENTS

Alhamdulilah, first and foremost, I am using this opportunity to express my gratitude for the aspiring guidance from my project supervisor, Assoc. Prof. Dr. Rahim. Without his assistance and dedicated involvement in every step throughout the process, this paper would have never been accomplished. I would also like to thank my project Co-supervisor, Assoc. Prof. Dr. Siti Khairani for always making time for me despite her other commitments. To Bacteriology laboratory staff, Miss Ada, Miss Kris and Mr. Azri, thank you for your guidance and always willing to help on anything. Thank you for spending your precious time teaching and waiting for us on the weekends and over working hours on the weekdays. Of course, not to forget, I would like to acknowledge my project partner, Tuan Ajmal for all your help and having to tolerate me for the past few weeks.

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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 project.

IDENTIFICATION OF POTENTIAL BACTERIAL PATHOGEN IN LABORATORY RATS PRODUCED LOCALLY

By

Nur Asyikin binti Haron

2015

Supervisor: Assoc. Prof. Dr. Abdul Rahim Mutalib

Microbiological status of laboratory rats used in experiments can significantly influence the animals' welfare and compromise research findings. Laboratory rats used in research must be healthy since any infection even in the absence of clinical signs before or during the experiment period may result in inaccurate data. In this study, 15 laboratory rats were chosen randomly from 3 different suppliers (5 rats from each supplier). The rats were euthanized and then, samples from the respiratory tract and gastrointestinal tract were collected using sterile swabs. Isolation and identification of bacteria from the samples were carried out by culturing followed by series of biochemical tests. Ten bacterial species were monitored in this study. They include *Staphylococcus aureus, Bordetella bronchoseptica, Pasteurella pneumotropica, Streptococcus pneumonia, Corynebacterium sp., Salmonella sp., Citrobacter sp., Clostridium sp., Pseudomonas*

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aeruginosa and Klebsiella pneumoniae. A number of bacterial species were identified, however, from the list mentioned, only *Pasteurella pneumotropica*, *Corynebacterium sp*., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were present from the samples. These bacteria are known as opportunistic pathogens where immunocompromised animals may succumb to infection. Animals used in research will undergo stressful conditions during experimental period which may eventually lead to immunosupression and thus presence of these opportunistic pathogens may compromise the research data.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901-Projek.

IDENTIFIKASI BAKTERIA BERPOTENSI PATOGENIK DALAM TIKUS MAKMAL KELUARAN TEMPATAN

Oleh

Nur Asyikin binti Haron

2015

Penyelia: Assoc. Prof. Dr. Abdul Rahim Mutalib

Status mikrobiologi tikus makmal yang digunakan dalam eksperimen boleh mempengaruhi kebajikan haiwan dan hasil penyelidikan dengan ketara. Tikus makmal yang digunakan dalam penyelidikan mestilah sihat kerana sebarang jangkitan walaupun tiada tanda-tanda klinikal sebelum atau dalam tempoh percubaan ia boleh mengganggu data yang di rekodkan. Dalam kajian ini, 15 ekor tikus makmal telah dipilih secara rawak daripada 3 pembekal yang berlainan (5 ekor tikus dari setiap pembekal) . Tikus tertakluk kepada eutanasia dan kemudian, sampel daripada saluran pernafasan dan saluran gastrousus diambil dengan menggunakan swab steril. Pengasingan dan pengenalpastian bakteria daripada sampel telah dijalankan melalui pengkulturan diikuti oleh ujian biokimia . Sepuluh spesies bakteria telah dipantau dalam kajian ini, termasuk *Staphylococcus aureus* , *Bordetella bronchoseptica* , *Pasteurella pneumotropica* , *Streptococcus pneumonia* , *Corynebacterium sp.* ,

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Salmonella sp., Citrobacter sp., Clostridium sp., Pseudomonas aeruginosa dan Klebsiella pneumonia. Beberapa spesies bakteria telah dikenal pasti, walaubagaimanapun, daripada senarai di atas, hanya *Pasteurella pneumotropica, Corynebacterium sp., Pseudomonas aeruginosa* dan *Klebsiella pneumoniae* di kenalpasti dari sampel. Bakteria-bakteria ini dikenali sebagai patogen oportunis dimana haiwan yang mempunyai system imuniti yang tidak kompeten boleh menjadi sakit. Haiwan yang digunakan dalam penyelidikan akan menlalui keadaan tertekan dalam tempoh eksperimen yang akhirnya boleh menyebabkan system imuniti di kompromi dan dengan itu kehadiran patogen oportunis ini boleh menjejaskan data penyelidikan

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1.0 INTRODUCTION

Laboratory rats rank second to mice in number used in biomedical research and both account for 90% of all mammalian species used (Hrapkiewicz & Medina, 2007). The characteristics of the rats make them valuable for research purposes. These include short life span, short gestation period, large litter size, great genetic diversity, affordable purchase price and ease of maintenance. Several areas for which rats are used in research are neoplasia, hypertension, teratology, toxicology, embryology and aging. In selecting laboratory animals for research, proper consideration should be given to the quality of the animal and health is one of the major qualities. It is vital that animals used in research are healthy. The microbiological status of these animals can influence the validity of the research data as any form of infection with certain microorganism can interfere with the research. Therefore, health monitoring plays an important role to detect potential pathogen in laboratory animals. In interpreting the microbiologic status of laboratory animals, it must be understood that infection is not synonymous with disease. Infection simply indicates the presence of microbes, which may be pathogens, opportunists, or commensals, of which the last two are most numerous (National research council, 1991).

Infectious agent may affect the host in various ways. Some agents are pathogenic, meaning that the agent have the capacity to cause disease in a

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susceptible host and may induce clinical signs. Other agents, cause no disease or only mild disease. In advanced cases, infection may also cause death. Animals used in research are undoubtedly undergoing tremendous amount of stress. Both mental and physical disturbance may cause stress but the latter is probably the most important in animals. Stress is often linked with the circumstances that contribute to the development of diseases. This is because, if the disturbance is severe enough, the body's autonomic nervous system or the pituitary-adrenal axis response will be activated. Thus, this will lead to the release of corticosteroids and catecholamines which function to counter the harmful effects of the disturbance. Corticosteroids in large amounts depress the inflammatory response, causes thymic involution and lymphocyte destruction which in turn may aggravate infection. Impairment of the host immune system leads to opportunistic infection caused by normally harmless commensals.

The use of laboratory animals that are free from unwanted organisms are significant to achieve reliable and reproducible result with minimum number of animals. Due to what has been described, it is crucial that animals used in research have a microbiological status that is known. However, it is difficult to assess the microbiological status of the individual taking part in the experiment. For that matter, the status of the colonies is usually taken into consideration rather than individual status. This is where health monitoring comes into play. The goal of health surveillance programs is to detect by examination of 1 or more representative sample groups for the presence (even in a single individual) of any pathogen from a specific profile of infectious agents. If an agent is detected in the sample group, the

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larger population (such as the breeding unit or research animal room) should be considered contaminated (or infected) with the same detected agent (Weisbroth et. al, 1998)

In this study, only bacteriological examination was carried out as a part of health monitoring program in laboratory rats. Five rats were sampled randomly from three different suppliers and ten bacterial species that have research-interfering potential were monitored which include *Staphylococcus aureus, Bordetella bronchoseptica, Pasteurella pneumotropica, Streptococcus pneumoniae, Corynebacterium sp., Salmonella sp., Citrobacter sp., Clostridium sp., Pseudomonas aeruginosa and Klebsiella pneumoniae.* Other than monitoring the listed bacteria, this study also record the ongoing microbiological status of the laboratory rats produced locally in Malaysia.

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2.0 LITERATURE REVIEW

As the health of laboratory animals have a major impact on their suitability for research, animal health monitoring program is vital in research facilities and also laboratory animal suppliers. In Europe, there are a few of recommendations published intended to standardize health monitoring program and thus help to standardize the microbiological quality if animals. Such programs are published by Federation of European Laboratory Animal Science Associations (FELASA) and also by renowned laboratory animal research facility such as Charles River Laboratories International Incorporated.

Microbiological standardization aims to produce animals that meet preset requirements of microbiological quality, aid in the maintenance of this quality during experiments and also act as a part of quality assurance system (FELASA, 2001). The result of health monitoring program must be recorded and summarized for clarity and ease of use. This information is used to monitor the microbiological status of the colonies. Routine microbiological surveillance of animals helps to prevent spreading of unwanted microorganisms in the colonies and help to determine whether the preventive measures used in the facility have been effective.

Microbiological assessment of the health status of laboratory rats is defined as the science of evaluating representative sample from given units against a specific listing of etiological agents of disease to define the health status of the source colony

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(Weisbroth et. al, 1998). Animal unit may have a higher risk to the introduction of unwanted microorganism if there are frequent introduction of animals for example, introduction of animal from different breeding colony, frequent entry of research personnel into the animal unit other than the animal care staff, or there are access for other animals to enter the unit. However, animal unit may have a lower risk if they practice “all in & all out” system (FELASA, 2014). Colonies should be monitored at least quarterly (FELASA, 2001). However, the health monitoring program should be adapted to individual and local needs, research objectives and local prevalence of agents. Sick and dead laboratory rats should be submitted for necropsy in addition to those already scheduled for routine monitoring (FELASA, 2001).

These are few terminologies for microbiological status of laboratory rats according to National Research Council in 1991

a. Axenic animals or also known as germ free animal are derived by hysterectomy and maintained in an isolator via germ free technique. These animals are free from all other life form which includes virus, bacteria, fungi, protozoa and others.

b. Gnotobiotic animals are also derived by hysterectomy n maintained in isolator by germ free technique. These animals are basically axenic animals that were inoculated with certain normal flora (Non-pathogenic agent)

c. Specific Pathogen Free animals are barrier maintained animals. As the name implies these animals are free from specified list of pathogenic organisms

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d. Conventional Animals are apparently healthy animal with unknown microbial burden. These animals are housed in open top cages

A diagnostic record of Taiwan National Laboratory center that represents 10% laboratory mice and rats from 2004 to 2007 reveals that there were several pathogens was identified by bacteriologic culture. The pathogens are such as *Bordetella bronchiseptica, Corynebacterium kutscheri, Pasteurella pneumotropica, Staphylococcus aureus, Streptococcus pneumoniae* from tracheal loop swab, *Citrobacter rodentium, Salmonella* spp. from caecal contents, and *Klebsiella pneumoniae* from both tracheal swab and caecal contents. In korea, *Corynebacterium kutscheri, Mycoplasma pulmonis, Pseudomonas aeruginosa* and *Pasteurella pneumotropica* are the pathogens that were detected in laboratory rats from 34 facilities by International Council for Laboratory Animal Science (ICLAS) monitoring subcenter in between 1999 to 2003.

The ICLAS proposed that the microbiological status of animal facilities was divided into five different categories. Category A consists of zoonotic and human pathogens carried by mice and rats; Category B comprise of fatal pathogens to mice and rats; Category C, potential pathogens capable of causing disease and affecting the physiological functions in mice and rats; Category D comprise of opportunistic pathogens of mice and rats; and finally, Category E consist of microbes as indicators of the microbiological status of mice and rats. Korea is one of the countries that follow this proposition.

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According to the health monitoring report March 2012 from Monash University Clayton campus where 5 to 6 rats were subjected for microbiological assessment. Presence of *Proteus sp.*, *Staphylococcus aureus* and *Staphylococcus spp*. (beta haemolytic) was reported. The prevalence of *Proteus sp*. in October 2011 was 100% where 6 out of 6 rats were tested positive. However, the prevalence of *Proteus sp*. decreases in March 2012 to 60%. This shows that health monitoring is important so that appropriate action can be taken in order to exclude the unwanted organism from the colonies.

Health monitoring is important to detect unwanted pathogens in the colony. Infection with these microorganisms may make it difficult to interpret the results in the experiment or leading to false interpretation. Other than that, it may increase variation inside the group and thus, leads to the usage of more animals in the study. Other than the environmental, nutritional, genetic and social influences factor, pathogen status has emerged as a crucial influence on host physiology. The selection of agents to be monitored is determined by numerous factors including effects on animal health, effects on biomedical research, species specificity, zoonotic potential, prevalence, host factors and desired unit microbiological status. There are limits to the utility of lists of agents to be monitored. First, such lists are never complete, as the list will change over time due to emergence and re-emergence of agents. The recommendations from FELASA and from major laboratory animal producers can be useful but the final decision about which agents to monitor at a facility has to be made by a veterinarian with specialization in laboratory animal medicine.

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3.0 MATERIALS AND METHOD

3.1 Animals

Five laboratory rats each were chosen randomly and sampled from three different suppliers, namely company A, B and C. The rats were at least 6 months old regardless of the sex from the breeding colony. The sampling was done at an interval between each company.

3.2 Sampling Procedure

The rats were euthanized by an overdose of carbon dioxide gas in an airtight chamber (Appendix A1). All five rats were placed in the same chamber and then the carbon dioxide (CO_2) was turned on. After about 30 seconds to 1 minute, the rats stopped moving and then the operator will check for the viability of the animals. The dead animals were then placed back into their carrier.

The instruments for dissection were dipped into 100% alcohol and left to dry each time prior to use. The wax tray was also cleaned with 100% alcohol before use. Sampling equipment comprised of sterile swab in a test tube, 10 sterile universal bottles-with 5ml of sterile saline, 5 bottles with 2ml of sterile saline and another 5 bottles with peptone water. The rat was placed on dorsal recumbency on the wax tray (Appendix A2) and the entire thoracic and abdominal area was disinfected with 70% alcohol to minimize contamination throughout sampling and to prevent the fur from contaminating the abdominal cavity. The first sample was taken from the nasal

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cavity where the skin around the nostrils was first removed using a sterile scissors to facilitate the sampling (Appendix A3). Sterile swab was introduced deep into both sides of the nasal cavity and then placed back into the glass tube carrier.

The skin of the abdominal cavity was incised along the midline from the pubis up to the mandible. The subcutaneous layer was undermined and the skin pulled laterally, away from the midline. Using a sterile scissors the muscle of the abdomen was incised carefully so as not to injure the abdominal viscera thus, opening the peritoneal cavity. The gastrointestinal tract was identified from the stomach until the rectum. Then, the caecum was incised and sterile swab was introduced into the caecum and placed into one of the sterile universal bottles with 5ml sterile saline. After that, the ileum of the intestines was identified and incised using sterile scissors about 1 inch in length. Sterile swab was introduced and pressed firmly against the intestinal mucosa along the incised length. The swab was then placed into the sterile universal bottle with 2ml sterile saline.

Faeces were collected by opening the rectum with sterile scissors. Few faecal pellets were placed into sterile universal bottle with peptone water for Salmonella sp. screening and another few pellets into sterile universal bottle with 5ml saline. The thoracic cavity was opened using sterile scissors to observe for signs of lesion and the appropriate sample was taken if there was any lesion present.

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3.3 Culture procedure

The samples in universal bottles were mixed using the vortex machine until the content was homogenous. A sterile wire loop was then dipped into the universal bottle containing the sample and the loop was streaked onto the blood agar. The procedure was repeated for MacConkey's agar. These procedures were repeated for all the samples in the universal bottles. The swab sample taken from the nasal cavity was directly streaked onto the blood agar and MacConkey's agars. The agar plates and the faeces sample in universal bottle with peptone water were then incubated at $37^{o}C$ for 24hours.

3.4 Gram staining from sample, primary culture and pure culture

Direct smear was done from all the samples collected. Two loops of sample was taken using the sterile loop and placed onto the glass slide from the samples in the universal bottle. The sample was then spread on the slide, air dried, then passed over the flame a few times to heat fixed it before gram staining was carried out. Gram staining was also repeated for the primary culture and pure culture. This is where a drop of normal saline was placed onto the glass slide and then half of a well isolated bacterial colony was taken, mixed with the saline and spread on the glass slide.

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Gram staining of the pure culture is to determine either the colony is of gram negative or gram positive bacteria. This procedure will help to determine the series of biochemical tests that need to be carried out in order to identify the bacteria species.

3.5 Examination of culture after 24 hours

The primary culture from the blood agar was examined and the colony morphology described. Selected colonies were gram stained and streaked onto a new blood agar for pure culture and incubated for 24 hours at 37^{o}C. The primary culture from the MacConkey's was described as lactose or non-lactose fermenters. The colonies were then gram stained. After that, the colonies were selected and also streaked onto a new blood agar for pure culture. The primary culture was then incubated for a further 24 hours for slow growing bacteria, if present, and then observed.

3.6 Isolation and identification of Salmonella sp.

As mentioned above, the faeces collected were placed into sterile universal bottles containing peptone water (pre-enrichment broth), mixed thoroughly and incubated at 37^{o}C for 24 hours (Appendix A4). After incubation, 100 micro litres from the pre-enrichment broth was drawn and inoculated into 10ml Rappaport broth and then incubated for 24 hours at 37^{o}C.

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After the incubation period, one loop of the broth was streaked onto Brilliant Green Agar (BGA) and XLD agar using a sterile loop. The agar plates were then incubated for 24 hours at 37^{o}C. The next day, the colonies on both agars were examined. Presence of pink colonies on BGA and pink colonies with black center on XLD agar was suggestive of *Salmonella sp.*

The suspected colonies were then streaked onto a new blood agar for pure culture and incubated for 24 hours at 37^{o}C. The pure cultures were tested using slide agglutination test using Salmonella Polyvalent "O" antiserum.

3.7 Biochemical test

Based on the gram staining characteristics of the pure cultures, either gram positive or gram negative, different sets of biochemical tests were used to identify the bacteria. A manual for the identification of bacterial species was used as a guide entitled, A diagnostic manual of veterinary clinical bacteriology and mycology written by Jang, Biberstein and Hirsh in 2008.

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Table 1: List of biochemical test

List of biochemical test		
Gram positive bacteria		**Gram negative bacteria**
Staphylococcus	Streptococcus	
Blood broth	Blood broth	Triple Sugar Iron (TSI)
Voges–Proskauer (VP) test	6.5% Nacl	Sulfide Indole Motility (SIM)
Maltose	Bile esculin	Citrate
Mannitol	Lactose	Urea
Alcohol dehydrogenase (ADH)	Sorbitol	
	Trehalose	
	ADH	

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4.0 RESULT

Table 2: Bacteria present in the respiratory and gastrointestinal tract

Bacteria	Rats from Company A		Rats from Company B		Rats from Company C	
	(n/5)	%	(n/5)	%	(n/5)	%
Staphylococcus intermedius	4	80	1	20	1	20
Escherichia coli	5	100	5	100	5	100
Pantoea agglomerans	2	40	0	0	1	20
Staphylococcus hyicus	1	20	4	80	0	0
*Pasteurella pneumotropica**	1	20	1	20	0	0
Proteus mirabilis	1	20	4	80	4	80
Aeromonas sp.	1	20	0	0	0	0
Enterococcus durans	1	20	0	0	0	0
*Corynebacterium sp.**	0	0	1	20	1	20
*Pseudomonas aeruginosa**	0	0	1	20	0	0
Edwardsiella tarda	0	0	1	20	0	0
*Klebsiella pneumoniae**	0	0	0	0	1	20
Actinomyces sp.	0	0	0	0	2	40
Bacillus cereus	0	0	0	0	1	20
Staphylococcus sp.	0	0	0	0	1	20
Streptococcus dysagalactiae	0	0	0	0	1	20
Morganella morganii	0	0	0	0	1	20
*Staphylococcus aureus**	0	0	0	0	0	0
*Bordetella bronchoseptica**	0	0	0	0	0	0
*Streptococcus pneumoniae**	0	0	0	0	0	0
*Salmonella sp.**	0	0	0	0	0	0
*Citrobacter sp.**	0	0	0	0	0	0
*Clostridium sp.**	0	0	0	0	0	0

(* Referring to the bacteria included in the monitoring program)

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Bacteria listed in Table 1 were isolated from the respiratory and gastrointestinal tract of laboratory rats from 3 different suppliers. From the list of bacteria monitored, only *Pasteurella pneumotropica, Corynebacterium sp.*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were detected. *Pasteurella pneumotropica* was isolated from Company A and B, where 20% were tested positive from both companies. *Corynebacterium sp.* was isolated from Company B and C also with positive result of 20%. *Pseudomonas aeruginosa* and *Klebsiella pneumonia* was isolated from Company B and Company C respectively with positive result of 20%. Other than the listed bacteria that were monitored, other bacteria were also detected. The presence of *Escherichia coli* is the highest among all others where the bacteria were present in all the rats from the three suppliers. This is followed by *Proteus mirabilis* where both Company B and Company C have a positive result of 80% compared to rats from Company A with only 20% tested positive.

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5.0 DISCUSSION

The laboratory rats from all the suppliers were of the conventional types. This means that the health status of this group of animals is not known. The rats housed in a system where no barriers are used to prevent microbial contamination of the colony. This study revealed that the laboratory rats produced locally have various infectious agents present both in the respiratory tract and also the gastrointestinal tract. The target of this screening program is to detect the presence of an organism in a group of animals by detecting its presence in one animal. If an agent is detected in the sample group, the larger population should be considered contaminated or infected with the same detected agent(s) (Weisbroth et al., 1998). The goal is not to find out the prevalence of the infection in the population: one positive individual means the result is positive for the population.

Based on International Laboratory Animal Resources Committee (ILAR) formula, at least 5 animals are required to be sampled from a colony. This theoretically results in 95% chance of detecting an infection if at least 50% of the population is infected. The formula also assumed that both sexes are infected at the same rate and the population size is more than 100 animals. After the detection of an organism the question that frequently arise are if and how an experiment might be affected.

Pasteurella pneumotropica was isolated from both Company A and Company B. *P. pneumotropica* infections are mostly latent and infections in rodents

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are mainly regarded as secondary to a primary agent. Stress, including experimental stress may trigger latent infection. The incidence of sudden deaths during inhalation of anaesthesia in rodents might be caused by *P. pneumotropica* infected animal. Transmission of this agent is mainly by horizontal transmission. However, Hansen (2000) reported that vertical transmission is also possible where intra-uterine infection have a prevalence of 60% to 70%. Following infection, the agent colonizes mainly the upper respiratory tract but no pathologic lesions are present in most cases (Baker, 2003). Immunosuppression facilitates the establishment of infection in the lungs. Natural infection of laboratory rats with this agent could compromise research involving dermal, enterohepatic, reproductive, and respiratory systems (Baker, 2003). Other studies may also be affected if the general health of the animal is compromised due to *P. pneumotropica* infection.

According to Hansen (2000), Corynebacterial systematic have not been studied thoroughly in laboratory animals, some Corynebacteria isolates may be difficult to identify to the species level. The laboratory rats from Company B and C were tested positive for *Corynebacterium sp*. However, the species could not be identified. Documented effects of *Corynebacterium sp.* on research are limited. One of the important Corynebacterium species is *Corynebacterium kutscheri*. Any procedure that could lead to immunosupression of the animal results in activation of *C. kutscheri* infection. This could compromise studies involving enterohepatic, respiratory and urinary system (Baker,2003). This is because in rats, abscesses caused by *C. kutscheri* infection usually develop in the lungs and extend to the

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pleura. Haematogenous spread also occur and accounts for all abscess formation in various organs.

Pseudomonas spp. is ubiquitous in the environment and may contaminate tap water. It may be isolated from respiratory, digestive and genital system of rats (Hansen, 2000). In this study, *P. aeruginosa* was isolated from the caecum. The importance of *P. aeruginosa* is as an opportunistic pathogen, where this agent may cause conjunctivitis and rhinitis, and under experimental condition, it may cause pneumonia and septicaemia in rats. Disease caused by this agent is usually observed in immunosuppressed or stressed animal. Infection may be due to poor hygienic condition for example from contaminated water used for drinking and cleaning. The effects of *P. aeruginosa* on research include early death following radiation, cyclophosphamide treatment or cold stress (National Research Council, 1991). Other effects are induction of pulmonary fibrosis (McIntosh et. al, 1992), increased production of cytokines (Schultz, 2001), altered fluid transport across lung epithelium (Pittet et. al, 1996), inhibition of protein synthesis in the liver (Schumann *et. al*, 1998) and many more.

Klebsiella pneumoniae was found in rats from company C. This agent is considered an opportunistic pathogen where it normally inhabits the intestinal tract of rats and reports of clinical disease in immunocompetent rats are very rare. Infection with *K. Pneumoniae* could interfere with studies involving several organ systems but most notably the endocrine, enterohepatic, hematopoietic, lymphoreticular, respiratory and urinary system. This is because, following infection,

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haematogenous spread takes place and allow focal formation of abscess in various organ.

Bacteria other than the ones mentioned above were also isolated from the laboratory rats. They include *Escherichia coli, Pantoea agglomerans, Staphylococcus intermedius, Staphylococcus hyicus, Proteus mirabilis, Edwardsiella tarda, Bacillus cereus, Actinomyces sp., Streptococcus dysagalactiae, Morganella morganii, Aeromonas sp.* and *Enterococcus durans.*

There are 100% positive results for the presence of *Escherichia coli* in all the samples from all three companies. This is not an abnormal finding since most rodents carry *Escherichia coli* as a normal inhabitant in the caecum and the large intestines. Most strains of *E.coli* are non-pathogenic but may cause disease in immunodeficient animal. There are pathogenic strains of *E.coli* however, to determine the strains, further test is required. In this study, no further tests were carried out. Nonetheless, pure cultures of all the *E.coli* strains isolated from the samples were preserved kept so that further tests can be done in the near future.

In this study, *Proteus mirabilis* was present in samples from all three companies. Company B and C have the highest number of positive samples where 4 out of 5 rats were tested positive. Proteus is also ubiquitous in nature, found in soil, water and sewage. It may cause urinary tract infections including pyelonephritis and also cause systemic disease in severely immunosuppressed animal. Isolation of Proteus sp. in the primary culture may overlap other bacteria due to its swarming

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characteristic on the blood agar. Due to that, other bacteria that are present in that pure culture cannot be subcultured and identified.

Pantoea agglomerans was isolated from laboratory rats from Company A and Company C where two rats were tested positive from Company A and only one rat tested positive from Company C. This bacterium is widely distributed in nature and has been isolated from various ecological niches including plants, water, soil, humans and animals (Deletoile et. al, 2009). It is known to cause opportunistic infection in immunocompromised patients. *Bacillus cereus* was isolated from rat from Company C. This agent has a wide distribution in nature and is frequently isolated from soil and growing plants. The isolation of these two agents in this study might be due to possible contamination of the bedding with soil.

Staphylococci are found worldwide in all animal species. Some staphylococci are host specific but spread between species should be expected (Hansen, 2000). The most important pathogen among this genus is *Staphylococcus aureus* that is found with high prevalence in most colonies of laboratory rodents. However, *Staph. aureus* was not isolated from any of the samples collected. The *Staphylococcus* species that was isolated are *Staphylococcus intermedius* and *Staphylococcus hyicus.*

Aeromonas sp. was only isolated in a rat from Company A. This agent may be isolated from the caecum, genitals and respiratory organs of laboratory animals. It is a part of mixed secondary flora in relation to wound infection and respiratory disease but have little importance as primary pathogens (Hansen, 2000). Next,

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Edwardsiella tarda was isolated from a rat from Company B. This agent can be isolated occasionally from the intestinal tract of animals. A few opportunistic infections of *E. tarda* have been reported (Carter and Wise, 2004). Actinomyces sp. which was isolated from 2 rats from Company A may cause pyogranulomatous inflammation with nodules in the skin, thorax, abdomen and even in the central nervous system (Hansen, 2000).

Morganella morganii is occasionally isolated from low number of individuals in colonies but no disease symptoms is known to be associated with this infection (Hansen, 2000). This agent was isolated from a rat in Company C. *Enterococcus durans* isolated from rat from Company A is ubiquitous in nature but occasionally occurs in the respiratory tract or gastrointestinal tract of rodents (Dietrich, 1996). Contamination of *E. durans* in immunosuppressed animals can manifest a disease. The last bacteria isolated were *Streptococcus dysagalactiae* and it was isolated from rat in Company C. This agent has been associated with subcutaneous, hepatic and abdominal abscesses in ICR Swiss mice (Percy and Barthold, 2007)

From the list of monitored bacterial species, Company B appeared to have more positive result compared to the other two companies. Laboratory rats from Company B were tested positive for *P. pneumotropica, Corynebacterium sp*. and *P. aeruginosa.* This finding implies that further progress in detection, control and elimination needs to be carried out in that company to ensure improved

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microbiological standardization of laboratory animals. This will result in a more reliable results of animal experiments with fewer number of animal needed.

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6.0 CONCLUSION

From this study, it shows that the laboratory rats produced by the local conventional animal facilities are contaminated with numerous infectious agents. In order to decrease or to exclude the infectious agents, regular health monitoring program should be carried out. This not only include microbiological assessment, other tests to detect virus, parasites and fungus should be carried out to ensure good quality laboratory animal produced.

Direct effects of infectious agents on experiments may lead to false conclusion or misinterpretation. The use of healthy laboratory animals is crucial to ensure reliable and reproducible result. This is important so that to reproducible results can be obtained with minimum number of animals and thus contribute to animal welfare.

The companies should also consider the environmental factor that could lead to contamination of the laboratory rats. Facility may be contaminated through exposure to wild animals, poor management and different activities undertaken at the facility as animal unit may be at a higher risk if there are frequent introduction of animals. Health monitoring program is only one of the steps, collective effort such as good biosecurity and good laboratory practice are important to ensure good quality and healthy laboratory animal produced.

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APPENDIX A

A1 : The airtight chamber for euthanasia

A2 : Rat dissection

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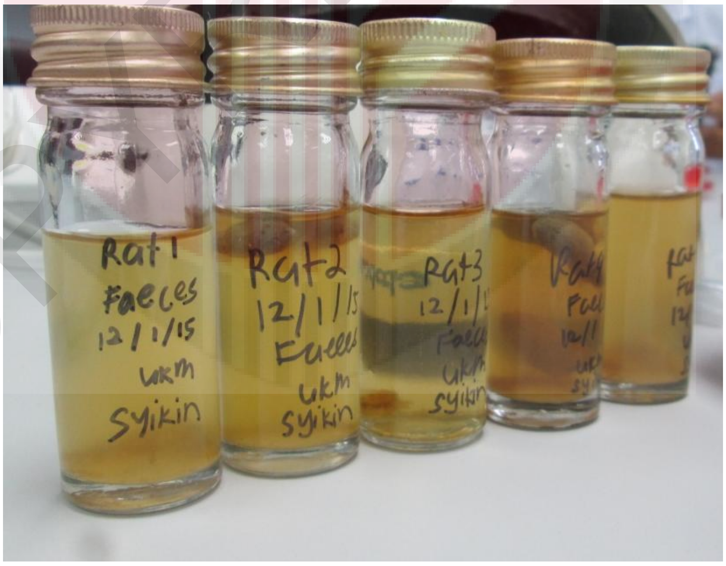
A 3: Sample collected from the nasal cavity using a sterile swab

A4: Faeces collected in sterile universal bottle with peptone water



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