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IDENTIFICATION OF BACTERIA FROM ORAL AND RECTAL SWABS FROM DIFFERENT SPECIES OF RODENTS IN KEMASUL FOREST RESERVE, PAHANG

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ABSTRACT

Small mammals such as rodents have potential in carrying disease caused by bacteria and this needs to be addressed to prevent serious disease outbreaks. Forest fragmentation by human activities adversely affected the flora and fauna diversity which indirectly increasing disease transmission to the living things in the forest and its surrounding. This research aims to determine the presence of bacteria in oral and rectal swabs of selected rodent species and to investigate the antibiotic susceptibility of the isolated bacteria. This study was conducted in Kemasul Forest Reserve, Pahang and trappings were conducted from August to November 2015. Swab samples were taken and bacterial identification was made in the laboratory by using biochemical test and Analytical Profile Index (API) kit. *Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter cloacae, Pseudomonas luteola, Corynebacterium kutscheri* and *Bacillus* spp. were identified, and there were no different bacteria species in the two different anatomical sites. These bacteria are normal commensal bacteria and opportunistic pathogens. Antibiotic susceptibility test indicated that ciprofloxacin, and vancomycin are the most susceptible antibiotic for all bacteria. This knowledge will enhance understanding of bacterial infection in rodents and becomes important baseline data for bacteria species.

Keywords: Bacterial identification, antibiotic susceptibility, disease transmission, oral and rectal swabs of rodents

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INTRODUCTION

Rodents are a group of mammals that are successfully exploiting diverse habitat and environment for their survival worldwide. Most rodent species live in the wild with less direct interaction with human being. There are some species found in the wild including *Maxomys rajah, Maxomys surifer, Leopoldamys sabanus, Sundamys muelleri* and *Hylomys suillus* (Mariana *et al*., 2008; Zain *et al*., 2015). Meanwhile, some rodents' species have adapted to live in urban areas close to human settlement such as *Rattus rattus, Rattus norvegicus, Maxomys whiteheadi, Mus musculus* and *Mus domesticus*. Sahimin *et al*. (2014) reported that *Rattus rattus diardii* is the dominant domestic rats in urban areas in Malaysia.

Previous studies by Castillo *et al*. (2003) reported that 74% of commensal rodents were captured in the urban areas. *M. domesticus* was the most abundant species followed by *Calomys musculinus* which was related to open areas such as railway bank, streams bank and rubbish dump. Furthermore, they are considered as pests that eat crops, animal droppings, garbage, and other rats. Additionally, rodents are well known as carriers of a variety of diseases that can infect both humans and livestock. Many of these rodent species are also reservoirs that cause debilitating diseases in human and livestock (Singleton *et al*., 2003). These rodents can spread and transmit disease as they live close to human habitat. Consequently, rodents can vector more than 60 known diseases (Phan *et al*., 2011), some of which are zoonotic diseases, caused by bacteria that pose a risk to human health and could lead to death. For instance, *Aeromonas hydrophila* bacteria can be transmitted from rodents to human which can cause gastroenteritis with diarrhoea symptoms (McCoy *et al*., 2010) and *Leptospira* spp. can cause death due to renal failure, cardio pulmonary failure and haemorrhage (Holt *et al*., 2006).

Kemasul Forest Reserve, located in Pahang, has been converted to monoculture plantation and leaving some forest remnants scattered within the forest. Opening areas for this project resulted in the separation of the forest into small fragments that caused disturbance to wildlife that lives in the forest fragments and forced

them to approach human settlements near the forest and resulting conflict between human and animal. These animals have undergone long series of physiological stress due to habitat disturbance and therefore easily infected with diseases. Furthermore, as human activities within the forest increase, there has been a growing concern if the natural environment has been adversely affected. Habitat disturbance may increase bacterial infection to the animals due to their poor health condition. As rodent are hosts and potentially be infested with bacteria, there are high possibilities that disease may present among these species and may cause deleterious effects to other living things surround them. Hence, the objectives of this study are to isolate and identify bacteria of different anatomical sites of rodents and to investigate the antibiotic resistance of the isolated bacteria.

METHODOLOGY

Study Areas

Kemasul Forest Reserve in Pahang (Figure 1) was selected as a case study. The study was conducted in two different locations which were Chemomoi and Jambu Rias. Latitude and longitude for Chemomoi are 03°19′34.2″N, 102°15′0.16″E and for Jambu Rias are 03°27′18.3″N, 102°08′175″E (Figure 2). Kemasul Forest Reserve has a total area of 39,000 hectares includes areas of Temerloh, Bera, and Bentong (Figure 2). The whole area was gazetted as Forest Farm project under the supervision of the Pahang Forestry Department. Forest Project was privatised in 1994.

Figure 1 Location of Kemasul FR in Pahang State in Peninsular Malaysia.

Figure 2 Jambu Rias (JR) and Chemomoi (CM) in Kemasul Forest Reserve, Pahang

Sampling Procedure in the Field

Trapping was employed from August to November 2015. Rodents were captured using small wire mesh cage traps. A total of 300 traps were deployed at the study sites for two weeks continuously for three months. Traps were placed above ground level, including on tree stumps, tree branches or fallen log. Baits used were oil palm fruits and jackfruits. The captured rodents were anaesthetised with about 0.5-1 mL of Zoletil (active ingredients; tiletamine and zolazepam). Then, weight and morphometric measurements of head and body, tail, ear, hindfoot and physical appearances were recorded. Each animal's age, sex, and species were identified following a guide authored by Francis (2008). Swab samples were obtained from the oral and rectal mucosa of each rodent using sterilised swabs for collection of bacteria. UKM Animal Ethics Committee approved all procedures with approval number FST/2016/SHUKOR/18-MAY/750-MAY-2016-SEPT.-2018-AR-CATS. All captured individuals were released at the point of capture after they gained consciousness. Only four rodents species were selected in this study namely *M. rajah, M. surifer, M. whiteheadi* and *R. rattus*. The first two species were forest species, while the later were commensal species near human settlements.

Bacterial Identification

Serial dilution method was used where a series of sequential dilutions used to reduce a dense bacteria solution. Swab sample of different species was dipped in 99 mL of sterilised saline solution and diluted till 10^{-6} dilution. A 1 mL pipette was used to transfer the sterilised saline contained culture into the 10^{-1} bottle. The bacterial suspension was mixed thoroughly using the vortex mixer. The volume of 0.1 mL of the final three dilutions (10^{-2}, 10^{-4}, and 10^{-6}) was transferred into the nutrient agar plates and was spread evenly over the entire surface of the nutrient agar plates until the medium no longer appears moist. The triplicate plates were incubated at 37˚C for 24 hours.

Next, streak plate method was used to obtain single colonies of bacteria for isolation of pure bacteria cultures (Sanders, 2012). The sample was picked and spread over about one-quarter of the surface of the medium and repeated until all four quarters were covered. The plate was labelled and incubated inversely at 37˚C for 24 hours. The colony forming units (CFU) for each plate were counted manually after incubation period was completed prior to observation.

One of the basic steps in identifying bacteria is observing its colony morphology such as colour, size, form, elevation, and margin. According to Chauhan (2012), Gram staining is an empirical method of differentiating bacterial species into two large groups of Gram-positive and Gram-negative bacteria. One single colony was placed on a clean glass slide. The smear was air-dried and heat fixed. The slide was treated with crystal violet for 1 minute followed by washing with running water. Then, iodine solution was used to cover the slide for 1 minute followed by washing. After that, the slide was treated with alcohol until decolourised. Finally, the slide was counterstained with safranin for 30 seconds followed by washing with running water. Then, the slide was air-dried and examined under a microscope to identify the morphology of bacteria.

Biochemical tests were used for the identification of bacteria species based on the differences in the biochemical activities. This test can distinguish the differences in carbohydrate metabolism, protein metabolism, fat metabolism, production of certain enzymes and ability to utilise a particular compound. The catalase test was primarily used for Gram-positive bacteria and can, for instance, be utilised to distinguish *Staphylococcus* spp. and *Micrococcus* spp. A small amount of bacterial colony was transferred onto the slide by using a sterile wooden stick. Then, a drop of H_2O_2 was placed on the slide and mixed. The oxidase test was used to determine if an organism possesses the cytochrome oxidase enzyme and currently use for differentiating Gram-negative rod either *Enterobacteriaceae* or

Aeromonas, Pseudomonas and *Vibrio*. A strip of filter paper was soaked with the substrate (1% tetramethyl-p-phenylenediamine dihydrochloride). Then, a speck of culture was rubbed on the filter paper.

A pure colony of bacteria was stabbed into Simmons Citrate Agar (SIM) agar for motility test. Citrate test was used to determine the ability of bacteria to utilise sodium citrate as its only carbon source and inorganic ($NH_4H_2PO_2$) is the sole fixed nitrogen source. Commonly it is used to identify Gram-negative pathogens of *Enterobacteriaceae* family. The colony was stabbed into the SIM on the slant and was incubated for 24 hours. Bacterial growth usually results in the bromothymol blue indicator, turning from green to blue. The bromothymol blue pH indicator is a deep forest green at neutral pH. With an increase in medium pH to above 7.6, bromothymol blue changes to blue (Acharya, 2013).

Next, the purpose of starch hydrolysis test was to investigate whether the microbe can use starch, a complex carbohydrate made from glucose, as a source of carbon or energy for growth. An inoculum from a pure culture was streaked on a sterile plate of starch agar. The inoculated plate was incubated at 37˚C for 24 hours. Iodine reagent was then added to submerge the growth. Mannitol Salt Agar (MSA) is a selective and differential medium used to isolate and differentiate species of salt-tolerant bacteria such as *Staphylococci* sp. The colony from pure culture was streaked and incubated for 24 hours.

Analytical Profile Index (API) Kit

An API test strip is a classification of bacteria in advance and quick identification. It contains series of cupules including various freeze-dried reagents and colour indicators designed for biochemical tests. The cupules were inoculated with a suspension of purified samples containing the microbe of interest, most incubated for 24 to 48 hours at 20˚C (Holmes *et al*., 1978). The results generated a code that can be entered into a database to identify the microbe of interest. API kit 20E was used in this study for identification of Gram-negative rods.

Antibiotic Susceptibility Test

Disc diffusion test or the Kirby-Bauer test was used for the antibiotic test. All bacterial isolates were tested for their susceptibility to 8 different antibiotics using the disk diffusion technique according to National Committee for Clinical Laboratory Standards. This test was used to identify the resistance or sensitivity of bacteria to specific chemicals so the best antibiotic treatment can be suggested

for patients infected by selected bacteria. The presence or absence of an inhibitory area around the disc indicated the bacterial sensitivity to the selected drug. If there is no growth around antibiotic discs, means the organism is killed or inhibited by the concentration of the antibiotic. Bacteria were inoculated onto Mueller Hinton broth and incubated overnight at 37˚C. The concentration of bacteria was standardised using Mc Farland standard. Then, sterile cotton swab dipped into bacterial suspension prepared and evenly spread on Mueller Hinton agar. The plate left to dry before proceeding with placing the antibiotic disc which are ampicillin, ciprofloxacin, kanamycin, neomycin, penicillin, polymyxin, streptomycin, and vancomycin onto the agar using a forcep. One filter paper disc with a diameter of 6 mm dipped into dimethyl sulfoxide (DMSO) served as a negative control also placed on the same plate. The plates were incubated at 37˚C for 24 hours. After that, the clear zone was observed and measured.

RESULTS AND DISCUSSION

Isolation and Identification of Bacteria in Different Anatomical Sites

A total of eight swab samples from two different anatomical sites of four different species of rodents were isolated. Table 1 showed the average CFU/mL of each plate with 30-300 colonies for four different species of rodents. The study revealed that the concentration of bacteria was higher in non-forest species compared to forest species as shown in *M. whiteheadi* and *R. rattus* with higher bacteria concentration in both anatomical sites. These were expected since the non-forest species were more exposed to approaching human settlements. Bacteria species, however can be transmitted between urban and forest rodents because they inhabit the same environment. Furthermore, most of the bacteria species were normal commensal bacteria such as *S. epidermidis*, *S. aureus* and *C. kutscheri*. However, *C. kutscheri* only found in the oral swabs of rodents. Similar to this study, Holmes and Korman (2007) stated that *C. kutscheri* is a common bacterium isolated from the oral cavity of rodents. This finding indicates that human disturbance to the Kemasul forest induces diseases' prevalence.

Habitat disturbance by humans is causing increased stress levels and resulting in poor diets for the animal (Debinski & Holt, 2000) as a fragmented forest is prone to resource depletion due to the isolation and size effect (Fahrig, 2003). Thus, this has led to rodents expending more time and effort foraging for food in nearby human settlement (Michaux, 2008). Consequently, they become more susceptible to bacterial infection due to exposure to water contamination, air

pollution and landfill from human settlement. Also, they are easily infected with disease due to stress (Peterson *et al*., 2013).

Table 1 Result of average bacterial colonies for each serial dilutions

Species of rodents	Serial dilution	Oral	Rectal
		Colonies/mL	Colonies/mL
Maxomys rajah	10^{-2}	8.30×10^{3}	3.90×10^{3}
Maxomys surifer	10^{-2}	6.30×10^{3}	7.90×10^{3}
Maxomys whiteheadi	10^{-2}	1.08×10^{4}	1.86×10^{4}
Rattus rattus	10^{-4}	1.71×10^{6}	2.31×10^{6}

After the isolation was completed, the morphology of Gram-positive bacteria for each colony on the plates was observed to characterise the bacteria as shown in Table 2. All samples indicated negative results for oxidase test when appearing colourless on the filter paper. Meanwhile, for catalase test, all samples showed the positive results when producing bubbles on the glass slides. This result directly showed the rapid elaboration of oxygen. The catalase test was primarily used for Gram-positive bacteria which are catalase positive from *Streptococcus* spp. and *Enterococcus* spp. respectively, which are catalase negative. For motility test, the positive result shows the growth of bacteria from the stabbed line which indicates the bacteria are motile. The organism is motile when it swims away from the line of inoculation into the uninoculated surrounding medium. While for starch hydrolysis test, the presence of clear halos surrounding the bacterial colonies shows the positive result for their ability to digest the starch and thus indicates the presence of α-amylase. After further test by streaking on MSA, the plate agar turned to yellow which indicated *S. aureus* and when the other plate which remains same colour indicated *S. epidermidis*.

On the other hand, API kit 20E was used for the sample that carried Gram-negative *Bacillus* spp. for further confirmation of bacteria as shown in Table 3. The oxidase test was performed to differentiate *Enterobacteriaceae* or *Aeromonas*, *Pseudomonas,* and *Vibrio* spp., and all Gram-negative rod showed negative results for oxidase test using the API kit 20E. Six different bacteria species were identified in the four rodent species after comparing all the results using the flowcharts from Bergey's manual.

Table 2 Identification of Gram-positive bacteria in two different anatomical sites using biochemical test

Sample	Morphology		Biochemical test					Final identification
	Form	Shape	Mannitol Salt Agar	Novobiocin Sensitivity	Motility	Spore - forming	Starch hydrolysis	
MS31-T1 (*Maxomy surifer*)	Round, Convex, White-grey	Spherical	-	+	-	-	-	*Staphylococcus epidermidis*
MS31-T2 (*Maxomys surifer*)	Round, Convex, White-golden	Spherical	+	-	-	-	-	*Staphylococcus aureus*
MR11-T1 (*Maxomys rajah)*	Round, Convex, White-golden	Spherical	+	-	-	-	-	*Staphylococcus aureus*
RR24-T2 (*Rattus rattus*)	Round, Convex, White golden	Spherical	+	-	-	-	-	*Staphylococcus aureus*
MR45-T2 (*Maxomys rajah*)	Circular, Entire, Yellow, Smooth	Rod	-	-	-	-	+	*Corynebacterium kutsceri*
MW39-T2 (*Maxomys whiteheadi*)	Circular, Entire, Yellow, Smooth	Rod	-	-	-	-	+	*Corynebacterium kutsceri*
MS40-T2 (*Maxomys surifer*)	Circular, Entire, Yellow	Rod	-	-	-	-	+	*Corynebacterium kutsceri*
RR24-R2 (*Rattus rattus*)	Circular	Rod	-	-	+	+	-	*Bacillus* spp.
RR21-T1 *(Rattus rattus*)	Circular	Rod	-	-	+	+	-	*Bacillus* spp.
MW37-T2 (*Maxomys whiteheadi)*	Sticky	Rod	-	-	-	+	-	*Bacillus* spp.
RR21-R2 (*Rattus rattus)*	Cream	Rod	-	-	+	+	-	*Bacillus* spp.

Table 3 Identification of Gram-negative bacteria in two different anatomical sites using the API kit 20E

Sample	Morphology	Shape	Oxidase	Catalase	Motility	Final identification
MW39-T4 (*Maxomys whiteheadi*)	Smooth, Entire	Rod	-	+	+	*Pseudomonas luteola*
MR45-R1 (*Maxomys rajah*)	Convex, Entire, Shiny colonies	Rod	-	+	+	*Enterobacter cloacae*
RR24-R3 (*Rattus rattus*)	Convex, Entire, Shiny colonies	Rod	-	+	+	*Enterobacter cloacae*
MS31-R2 (*Maxomys surifer*)	Convex, Entire, Shiny colonies	Rod	-	+	+	*Enterobacter cloacae*
RR24-R1 (*Rattus rattus*)	Smooth, Entire	Rod	-	+	+	*Pseudomonas luteola*

As shown in Table 4, forest rodent species namely the *M. rajah* and *M. surifer* carried almost similar bacteria in different anatomical sites. From oral samples, *M. rajah* carried *S. aureus*, *E. cloacae* and *C. kutscheri* meanwhile, *M. surifer* carried *S. epidermidis*, *S. aureus* and *C. kutscheri*. Rectal sample on the other hand, showed *M. rajah* carried *S. aureus*, *E. cloacae* and *Bacillus* spp. meanwhile *M. surifer* carried *P. luteola* and *Bacillus* spp. In contrast, the different types of *Bacillus* spp. were found among the urban rodent species. The Gram-negative enteric bacilli such as *P. luteola* and *E. cloacae* also found in all urban species, whereby *S. aureus* was only carried by *R. rattus* found in oral swabs. Obviously, *C. kutscheri* only found in the throat of these two different species which are *M. rajah* and *M. surifer*.

Holmes and Korman (2007) stated that *C. kutscheri* is a common bacteria isolated from the oral cavity of rodents. This is further confirmed by the high detection rate of *C. kutscheri* from the oral cavity of infected rodents reported by Amao *et al*. (1995). This study showed that these bacteria were found in forest species compared to urban species. This shows that *C. kutscheri* infection is high in urban rodents' species e.g. *Rattus* sp. *C. kutscheri* had been reported in *Mastomys natalensis* also known as Natal multimammate mouse, after 16S rRNA sequencing (Dassi *et al*., 2015). In the case of several bacteria such as *S. epidermidis, S. aureus,* and *P. luteola* that rarely infected rodents, there were some possible reason. As there were not many previous studies showed these bacteria

can infect rodents, the possible reason might be due to the destruction of their habitat. Habitat disturbance may increase bacterial infection to the animal due to a poor health condition and movement of the animal to nearest human settlement (Jones *et al*., 2013). It can cause indirect transmission of microbes through the physical environment, such as through contaminated soil or water, rather than transmission by direct contact. Rodents might get infected when they forage for food near human settlements as their natural habitat was being destructed and thus reduce in food sources. However, current study shows these bacteria did not give deleterious effect leading to death for rodents and human because all bacteria are normal bacteria flora that commonly found in human. The common disease of this bacterium can be treated by a commensal antibiotic.

Table 4 Comparison of bacteria between different species of rodent in two different anatomical sites

	Rodent species	Throat	Rectal
Forest species	*Maxomys rajah*	1. *Staphylococcus aureus* 2. *Enterobacter cloacae* 3. *Corynebacterium kutscheri*	1. *Staphylococcus aureus* 2. *Enterobacter cloacae* 3. *Bacillus* spp.
	Maxomys surifer	1. *Staphylococcus epidermidis* 2. *Staphylococcus aureus* 3. *Corynebacterium kutscheri*	1. *Pseudomonas luteola* 2. *Bacillus* spp.
Urban species	*Maxomys whiteheadi*	1. *Pseudomonas luteola* 2. *Bacillus* spp. 3. *Enterobacter cloacae* 4. *Corynebacterium kutscheri*	1. *Bacillus* spp. 2. *Bacillus* spp. 3. *Enterobacter cloacae*
	Rattus rattus	1. *Bacillus* spp. 2. *Staphylococcus aureus* 3. *Bacillus* spp. 4. *Bacillus* spp.	1. *Bacillus* spp. 2. *Enterobacter cloacae*

Rodents and human infections

Rodents can cause various diseases due to destruction, indirect contact with other animals and food contamination. A few potential rodent zoonoses diseases infected by bacteria were Leptospirosis, Campylobacter, Lymphocytic choriomeningitis (LCM) and other bacterial diseases. *C. kutscheri* is an infectious agent that is hazardous to rodent health, which can cause respiratory disease such as Pseudotuberculosis or Corynebacteriosis in these animals (Benato,

2012). This bacterium can be transmitted by direct contact, aerosol droplet, and faeces. The infections are usually inapparent and animals will consequently develop overt disease. The clinical signs usually include nutritional deficiencies, dyspnea with abnormal lung sounds, weight loss, rough hair coat, abnormal gait, swollen joints, humped posture and anorexia (Fox *et al*., 1987). In addition, the skin will be ulcerated, underlaid with fistulous tracts and abscessed in cutaneous infections (Funke *et al*., 1997). The previous study by Amoa *et al*. (2002) reported that healthy rat usually could be infected by this disease which inhabited the oral cavity, esophagus, cecal contents, colon, and rectum. Holmes and Korman (2007) documented a case of human *C. kutscheri* infection following a rat bite using conventional biochemical tests and confirmed by 16S rRNA gene sequence analysis. Thus, although this disease is rare in human, precautions should be taken to prevent this bacterium from infecting human. Furthermore, *C. kutscheri* is considered as an important causative microorganism in the spectrum of rat bite-associated bacterial disease in humans (Abrahamian & Goldstein, 2011).

E. cloacae is known as an opportunistic pathogen that is not a primary human pathogen but can be an important cause of nosocomial infections (Keller *et al*., 1998). This bacterium is commonly found in water, sewage, soil, and faeces and known as opportunistic pathogens (Mezzatesta *et al*., 2012). This bacterium can infect wounds, urinary and respiratory tract and also cause blood and brain infections especially in immune-compromised individuals (Fata *et al*., 1996). Conversely, *E. cloacae* does not infect nor cause fatality in rodents, but rodents may act as a reservoir and potentially transmit disease to human. However, the combination of a *Bacteroides bivius* and *E. cloacae* has resulted in high abscesses in female Sprague-Dawley rats (Martens *et al*., 1993).

P. luteola is an opportunistic pathogen and is rarely pathogenic to humans. *Pseudomonas* sp. infections can develop in multiple anatomic locations in human, including skin, subcutaneous tissue, and bone, ears, eyes, urinary tract, and heart valves. Reports of infection caused by *P. luteola* are rare in human and rodents, but infection in immunocompromised human patients is common (Arnold *et al*., 2004).

S. epidermidis is the commensal flora on human and animal skin. This bacterium is an opportunistic pathogen. *S. epidermidis* contribute to conjunctivitis, keratitis or hair follicles on the edge of the eyelid called as folliculitis. *S. epidermidis* strains, usually do not cause infection in human as Akinkunmi *et al*. (2014) found *S. epidermidis,* a pathogenic strains of rodents isolated from the faeces

of healthy children. However, this bacterium can cause infections in people with a suppressed immune system. In contrast, there is a lack of information on direct infection in rodents, but recently, Chaudhry and Patil (2016) found a closer phylogenetic relationship of rice seeds to rodents, compared to human, suggesting evolution and lifestyle adaptation of plants and rodents. On the other hand, *S. aureus* is considered as one of the more important causes of naturally occurring skin lesions in rodents and humans compared to *S. epidermidis*. The bacterium is commonly found in fur, nasopharynx, lower digestive tract and skin of rodents. The clinical signs of infected rodents include eczematous lesions that occur on shoulders and necks other than orbital and facial abscesses, preputial gland abscesses, tail lesions and self-mutilation of the penis. *S. aureus* strains also produce enzymes and toxins that likely cause or increase the severity of other diseases such as food poisoning, septic shock, toxic shock syndrome and scalded skin syndrome (Kaneko & Kamio, 2004).

Antibiotics Susceptibility Test

Table 5 showed the results of inhibition zone diameters in different antibiotic against different bacteria. Five species of bacteria including *E. cloacae, P. luteola, S. epidermidis, S. aureus* and *C. kutsceri* were tested in this study. Mimoz *et al*. (2000) discovered that *E. cloacae* NOR-1 in the rat are resistant to imipenem but susceptible to cefepime and ceftazidime. Besides that, the bacterium is susceptible to penicillin, tobramycin, gentamicin, tetracycline, pristinamycin, fusidic acid, vancomycin and oxacillin as reported by Renaud *et al*. (2001). In this study, we found that *E. cloacae* is only susceptible to ciprofloxacin and vancomycin. Penicillin has been widely used to treat *S. aureus* but nowadays 80% of all *S. aureus* strains are resistant to penicillin. The bacterium strains acquired a genetic element coding for β-lactamase production (Stark, 2013). The previous study by Hafiz *et al*. (2002) reported that *S. epidermidis* is 100% resistant towards ampicillin and penicillin as reported in this study, where isolates of *S. epidermidis* from rodents were susceptible towards all antibiotic except penicillin. These results are in contrast with findings reported by Haque *et al*. (2009) that show the sample isolates are resistant to penicillin and ciprofloxacin. *E. cloacae* have an intrinsic resistance to ampicillin, amoxicillin, first-generation cephalosporins and cefoxitin owing to the production of constitutive AmpC β-lactamase (Davin-Regli & Pagès, 2015). Overall the outcome of the antibiotic susceptible test in this study indicates that only ciprofloxacin and vancomycin antibiotics can be effectively used for the treatment of diseases caused by the bacteria investigated.

Table 5 Inhibition zone diameters of different antibiotic against different bacteria

Bacteria	Diameter of inhibition zone (mm)								
	Ampicillin (10µg)	Ciprofloxacin (5µg)	Kanamycin (30µg)	Neomycin (50µg)	Penicillin (10µg)	Polymyxin (30µg)	Streptomycin (10µg)	Vancomycin (30µg)	DMSO
Enterobacter cloacae	14 (I)	30 (S)	21 (R)	17 (R)	17 (R)	10 (R)	18 (R)	17 (S)	-
Pseudomonas luteola	11 (R)	30 (S)	20 (R)	16 (R)	22 (R)	10 (R)	15 (R)	12 (S)	-
Staphylococcus epidermidis	16 (R)	27 (S)	22 (R)	16 (R)	27 (R)	12 (S)	16 (R)	19 (S)	-
Staphylococcus aureus	8 (R)	21 (S)	22 (R)	16 (R)	11 (R)	11 (R)	15 (R)	16 (S)	-
Corynebacterium kutsceri	16 (R)	27 (S)	22 (R)	16 (R)	27 (S)	12 (S)	16 (R)	19 (S)	-

I-intermediate S- Susceptible R-Resistant

CONCLUSION

The occurrence of bacteria in *M. rajah, M. surifer,* and *R. rattus* were successfully identified including *S. aureus, S. epidermidis, E. cloacae, P. luteola, C. kutscheri* and *Bacillus spp*. There were no differences of bacteria species between oral and rectal of rodents. However, *C. kutscheri* was only found in throat of *M. rajah* and *M. surifer*. Furthermore, most of the bacteria species were normal commensal bacteria such as *S. epidermidis*, *S. aureus* and *C. kutscheri*. This demonstrates that rodents have chances to be infected by bacteria when exposed to contaminated soil, food, and water which play an essential role as sources of bacterial infection. Finally, antibiotic susceptibility testing indicated that only two antibiotics tested can be used for treatment, whereby many antibiotics were found to be resistant to these bacteria.

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