ANTIMICROBIAL SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM RED–TAILDED RACERS (*Gonyosoma oxycephalum*)

LYDIA POW KAR MEN

DEPARTMENT OF INFECTIOUS DISEASES AND VETERINARY PUBLIC HEALTH
FACULTY OF VETERINARY MEDICINE
IPB UNIVERSITY
BOGOR
2019
1. Dilarang mengubah sebogak aturan seluruh koraga lurik di laboratorium penelitian hortikultura.

2. Dilarang memanuhrkan dan memproduksi sebogak atas seluruh koraga lurik di laboratorium penelitian hortikultura.

3. Penguji penelitian harus mengetahui dan menghormati aturan seluruh koraga lurik di laboratorium penelitian hortikultura.

Hak Cipta Dilindungi Undang-Undang
I hereby state that the thesis titled Antimicrobial Susceptibility of Coagulase Negative Staphylococci Isolated from Red-tailed Racers (Gonyosoma oxycephalum) is my original work with guidance from my lecturer advisors and has not been submitted in any form to any institutes of higher learning. The sources of information derived or quoted from published and unpublished works of other authors have been mentioned in the text and compiled under References at the end of this paper.

With this, I hereby transfer the copyright as author of this paper to IPB University.

Bogor, May 2019

Lydia Pow Kar Men
NIM B04158006
1. Dilengkapi sampel di daerah dengan daerah yang sama dan kualitas beras yang sama.
2. Dilengkapi sampel dengan daerah yang bersifat berbeda dan kualitas beras yang berbeda.
3. Penuhi persyaratan kualitas beras yang bersifat berbeda dan kualitas beras yang berbeda.
4. Persyaratan kualitas beras yang bersifat berbeda dan kualitas beras yang berbeda.
LYDIA POW KAR MEN. Antimicrobial Susceptibility of Coagulase Negative Staphylococci Isolated from Red-tailed Racers (*Gonyosoma oxycephalum*). Supervised by USAMAH AFIFF and DENI NOVIANA

Wild snakes are being poached in increasing numbers to meet the public demand of exotic pets. Red–tailed racers (*Gonyosoma oxycephalum*) are a species of snake that is commonly caught to be kept as pets in Indonesia. Coagulase negative staphylococci (CoNS) are often found on the skin and mucous membrane of reptiles. Usually, they rarely cause primary disease and infection in humans and animals, unless present in large amounts. However, CoNS is emerging as an important human and animal pathogen. Antibiotic resistant CoNS has been reported around the world. Therefore, the aim of the study is to determine the antimicrobial susceptibility of CoNS isolated from red-tailed racers. Samples were swabbed from the oral cavity of 5 wild caught red–tailed racers, and were identified with biochemical test. The resistance test was done using the Kirby–Bauer disc diffusion test. The results were taken by measuring the diameter of the inhibition zone and interpreted by referring to Clinical and Laboratory Standards Institute (CLSI 2013).

Eight samples of bacteria isolated from the snakes were identified to be CoNS. Seven of the eight CoNS which consist of *Staphylococcus xylosus*, *Staphylococcus sciuri*, and *Staphylococcus lentus* show susceptibility to amoxicillin, gentamicin, erythromycin, bacitracin, vancomycin and oxacillin, but resistance towards penicillin G. One sample of *Staphylococcus sciuri* is intermediate towards erythromycin, and one sample of *Staphylococcus kloosii* show susceptibility towards amoxicillin, gentamicin, bacitracin, penicillin G, vancomycin, and oxacillin, but is resistant towards erythromycin.

Keywords: antimicrobial susceptibility, coagulase negative staphylococci, red–tailed racers, *Gonyosoma oxycephalum*
2. Diterima memuunkumunukon dan memperbanyoruk sembogkum oko seluukh koruka tuluk in dokom bentuk ogapun tompa izin IPB.

3. Penukupun kobor merugikum repurtukan young wolkor IPB.


1. Diterima memuunkumunukon oko seluukh koruka tuluk in tompan memuunkumunukon enam penulikan penelitian sembogkum.

Hak Cipta Dilindungi Undang-Undang
LYDIA POW KAR MEN. Kerentanan Antimikroba Staphylococci Koagulase Negatif Terisolasi dari Ular Bajing (Gonyosoma oxycephalum). Dibimbing oleh USAMAH AFIF dan DENI NOVIANA


Kata Kunci: Kerentanan antimikroba, Staphylococci koagulase negatif, ular bajing, Gonyosoma oxycephalum

2. Penurunan litar meturgi kripari dan penurunan yung. Ii: 


Hak Cipta Dilirinding. Linding-Linding

Bogor Agricultural Institute (Institut Pertanian Bogor)
Research Thesis
As one of the requirements of research for
Faculty of Veterinary Medicine

DEPARTMENT OF INFECTIOUS DISEASES AND VETERINARY PUBLIC HEALTH
FACULTY OF VETERINARY MEDICINE
IPB UNIVERSITY
BOGOR
2019
1. Dilengkapi informasi dan kontak person terkait proyek ini.

2. Dilengkapi informasi dan kontak person terkait proyek ini.

b. Proposal kegiatan yang wajar

c. Pendanaan kegiatan yang wajar

d. Penelitian kegiatan yang wajar

Hak Cipta Dilindungi Undang-Undang
Title: Antimicrobial Susceptibility of Coagulase Negative Staphylococci Isolated from Red-tailed Racers (*Gonyosoma oxycephalum*).

Name: Lydia Pow Kar Men

NIM: B04158006

Approved by

[Signature]

Advisor 1

[Signature]

Advisor 2

Acknowledged by

[Signature]

Prof. Drh. Agus Setiyono, MS Ph.D APVet

Vice Dean of Student and Academic Affairs

Faculty of Veterinary Medicine IPB

Date of Approval: 06 JUL 2019
1. Diterima mengisi spasi-sonon dalam hal yang harus diisi dalam formulir keuangan. Pemulihan untuk menanggung biaya pembuatan.

2. Diterima mengisi formulir-dana seluruh yang harus diisi dalam hal penggunaan yang wajar.

3. Diterima mengisi formulir-dana seluruh yang harus diisi dalam hal penggunaan yang wajar.
FOREWORD

Throughout this study, I have been deeply grateful to God for ensuring my good health and well-being to be able to carry out all my tasks. I would like to express immense gratitude towards both of my supervising lecturers, Prof. Drh Deni Noviana, PhD and Drh. Usamah Aiff, M.Sc for sharing their knowledge and experience for the development of this study as well as their continued guidance through writing this paper.

I would also like to thank Pak Ismet and all the staff members of the Department of Infectious Diseases and Veterinary Public Health for their help in preparing the necessary laboratory equipment to carry out this study. I want to take this opportunity to thank my friends Crystal Chang, Ang Jia Leng, Nike Choo Lee-An, Sohanjit Singh Chehal, Malarvily al/p Vasu and Dachainii a/p Theeran for their support and advice in carrying out this study.

Lastly, I would like to thank my family in Malaysia that have continually encouraged me from afar. May this research paper be beneficial to many and contribute towards the education and awareness of antibiotic resistance.

Bogor, May 2019

Lydia Pow Kar Men
2. Dilengkapi formulir dengan memperbaharui sebagian atau seluruh komponen yang diperlukan.

3. Formulir harus diperbaharui untuk mendaftarkan penelitian baru. Penelitian baru yang ditambahkan dalam formulir harus disertai dengan penjelasan singkat mengenai tujuannya.

4. Formulir harus diserahkan ke pihak yang relevan dalam waktu yang ditentukan.

---

Hak Cipta Didedikasi untuk IPB (Institut Pertanian Bogor)
INTRODUCTION

Background

The red–tailed racer, scientifically known as *Gonyosoma oxycephalum*, and also commonly known as red–tailed green ratsnake, is a species of semi arboREAL snake that is commonly found in Indonesia. It is heavily exploited in Java for its skin and meat, and is opportunistically exploited throughout Indonesia for the pet trade, skins, and for sale in restaurants. In the Indonesia pet trade, there is a particular demand for a grey and yellow colour morph known from Central Java. (Wogan et al. 2012). Due to the increase in demand of pet snakes, including red–tailed racers, many have turned to poaching wild snakes to meet public demand. However, not much is known about the antimicrobial susceptibility of bacterial flora of wild caught snakes, and the consequences it poses to public health.

One of the most commonly isolated bacterial sample from snakes are coagulase negative Staphylococci (CoNS). CoNS are commensal flora of reptile skin and mucous membrane, and rarely cause primary disease. However, the bacteria can cause infections when present in large amounts, or when present in the bloodstream. Among patients with blood cultures positive for CoNS, the fraction with a significant bloodstream infection ranges from 12 to 25 percent of cases (Souvenir et al. 1998).

Treating CoNS infections are traditionally difficult because many bacterial strains have become resistant to antibiotics. The World Health Organization (WHO) has named the emergence of resistance among bacteria as one of the three most important public health threat of the 21st century. Not only are they present in hospital environment, but now have been identified in community settings. This response by bacteria to antibiotics is the prime example of adaptation. The use of antibiotics by many countries has reached an excessive level and many are used inappropriately. Infections by multidrug–resistant (MDR) bacteria are associated with increased mortality compared to infections caused by susceptible bacteria (Munita and Arias 2016).

Purpose of Research

The purpose of this in–vitro study was to study the antimicrobial susceptibility of coagulase negative Staphylococci isolated from red–tailed racers (*Gonyosoma oxycephalum*) using the agar disc diffusion test.

Benefit of Research

The expected benefit of the research was to obtain information about the antimicrobial susceptibility of coagulase negative Staphylococci isolated from red–tailed racers (*Gonyosoma oxycephalum*).
Research Hypothesis

The coagulase negative Staphyloccci isolated from red–tailed racers (Gonyosoma oxycephalum) is sensitive to amoxicillin, penicillin G, oxacillin, gentamicin, erythromycin, bacitracin, and vancomycin.

LITERATURE REVIEW

Red – tailed Racer (Gonyosoma oxycephalum)

Kingdom : Animalia
Phylum : Chordata
Class : Reptilia
Order : Squamata
Family : Colubridae
Genus : Gonyosoma
Species : oxycephalum

Red–tailed racer, also commonly known as the red–tailed green ratsnake is a semi–arboreal snake, which distribution ranges from Myanmar eastward to central Viet Nam, southward through the Malay Peninsula and in island Southeast Asia, as far east as the Philippines and Indonesia. Their habitat is lowland tropical forest including riverine forest, bamboo forest, mangrove forest, and swamps. Red–tailed racers are long and slender, with adults having a bright green body dorsally and yellow–green ventrally. Their tail can be either reddish–brown, orange, or gray. Red–tailed racers feed mostly on birds, their eggs and their nestlings. They will also eat other small reptiles such as frogs and lizards, and other small mammals. They are not venomous and kill their prey through constriction and suffocation. This species of snake is harvested for the international pet trade, and is exploited in certain regions for skin and meat (Wogan et al. 2012).

Coagulase – Negative Staphylococci (CoNS)

CoNS are gram positive coci that divide in irregular “grape–like” clusters and are differentiated from S. aureus by their inability to produce coagulase and coagulate rabbit plasma. Species of CoNS that have important traits and are more frequently associated with clinical disease are S. epidermidis (biomaterial–based and prostatic device infections), S. lugdunensis (skin and soft–tissue infections, bacteremia, endocarditis), S. saprophyticus (uncomplicated urinary tract infections in sexually active women), and S. haemolyticus (often less–susceptible to vancomycin) (Becker et al. 2014).

Compared to S aureus, less is known about the virulence mechanism in CoNS, except for aspects of biofilm formation by S. epidermidis. Host susceptibility plays an important role in infection, because CoNS isolates lack the
virulence determinants responsible for aggression. Colonization of different parts of the skin and mucous membrane of the host is the key source of endogenous infection by CoNS. The first step in establishing colonization or infection by staphylococci is adherence to host. Colonization occurs by formation of a multilayered biofilm. The bacteria will first attach to the host, then multiply and accumulate in multilayered cell aggregates. This step requiring intercellular adhesion. Then it grows and mature into a thick, structured layer. Finally, single cells or cell agglomerates can dissociate from the biofilm and disseminate via the bloodstream to begin colonization and formation of biofilm at a different site, leading to metastasis of infection. In the biofilm, huge bacterial cell agglomerates are encased in an amorphous extracellular material composed of bacterial products, for example, teichoic acids, proteins, polysaccharides, and extracellular DNA (eDNA), and host product. It also contains channels that are fluid filled to ensure the delivery of oxygen and nutrients to the bacterial cells located in the deeper layers of the biofilm. *S. epidermidis* produces cytolytic toxins, such as the δ-toxin (PSMγ), which acts by forming pores in the cell membrane, leading to the lysis of erythrocytes and other mammalian cells (Becker *et al.* 2014).

Therapeutically, CoNS are challenging due to the large proportion of methicillin–resistant strains. Most infections by the *S. epidermidis* group require treatment with glycopeptides, especially vancomycin. However, β-lactamase-resistant penicillins and first or second generation cephalosporins is advisable for methicillin-susceptible isolates. Alternatively, newer antibiotic agents such as dapryanycin, linezolid or cephalosporin with methicillin-resistant *Staphylococcus aureus* (MRSA) activity, may be used if methicillin resistant is probable or was detected. For uncomplicated urinary tract infections caused by *S. saprophyticus* subsp. *saprophyticus*, cotrimoxazole can be administered. It is important to note that in cases of *S. lugdunensis* caused endocarditis, medical therapy is rarely successful and urgent surgical intervention is required. Drastic changes in patient populations—increased numbers of premature newborns and of elderly, multimorbid, chronically ill, and, often, immunocompromised patients—as well as the increasing use of inserted foreign bodies, led to an acknowledgment of the large variety of infections caused by CoNS (Becker *et al.* 2014).

As of 2014, the genus *Staphylococcus* consist of 47 species and 23 subspecies. Of these, 38 fulfil the categorization of a coagulase – negative species, and one further species, *S. schleiferi*, includes both a coagulase–negative subspecies (*S. schleiferi* subsp. *schleiferi*) and a coagulase–positive subspecies (*S. schleiferi* subsp. *coagulans*). A study done by Dehghani *et al.* (2015), shows that the highest rate of infection in the oral cavity of snakes from Iran is CoNS with 34.5%. Species of CoNS that have been isolated from the oral cavity of snakes include *Staphylococcus xylosus, Staphylococcus sciuri, Staphylococcus kloosii, Staphylococcus warner* (Artavia–Leon *et al.* 2017), and *Staphylococcus lentus* (Park *et al.* 2011).

**Mechanism of Antibiotic Resistance**

There are three fundamental mechanisms of antimicrobial resistance: (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that
Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome. The most important mechanism of resistance to the penicillins is antibiotic hydrolysis mediated by the bacterial enzyme beta-lactamase. The expression of chromosomal beta-lactamase can either be induced or stably depressed by exposure to beta-lactam drugs. Resistance to methicillin, which is stable to gram-positive beta-lactamase, occurs through the alteration of an antibiotic target protein, penicillin-binding protein 2. Production of antibiotic-modifying enzymes and synthesis of antibiotic-insensitive bacterial targets are the primary resistance mechanisms for the other classes of antibiotics, including aminoglycosides. Reduced antibiotic penetration is also a resistance mechanism for several classes of antibiotics, including the beta-lactam drugs and aminoglycosides (Dever and Dermody 1991).

The resistance genes found in multiply resistant CoNS, are the same as those found in S. aureus. Although majority of CoNS remain susceptible to vancomycin, isolates with reduced susceptibility have been observed. Reduced susceptibility to teicoplanin is observed in about 30% of S. haemolyticus and more rarely in S. epidermidis. The mechanism of glycopeptide intermediate susceptibility in S. epidermidis is the same as glycopeptide–intermediate S. aureus (Witte W 1999).

**Agar Disc Diffusion Test**

Disc diffusion method, also known as the Kirby–Bauer test is a standardized testing method for antimicrobial susceptibility. It is one of the most widely used methods for determining antimicrobial resistance due to its convenience, efficiency and relatively cheap cost. A standardized inoculum is swabbed on the surface of the appropriate agar. Fresh subcultures should be used because the reproducibility depends on the growth phase of the bacteria. Filter paper discs containing a standardized concentration of antimicrobial agent are placed on the surface and the agar is incubated overnight. Specific incubation time ranges are outlined in the Clinical Laboratory Standards Institute (CLSI) documents. After incubation, the size of the inhibition zone around the disc is measured to the nearest millimetre. Critical steps that should be taken into account is the medium used, depth and moisture content of the agar in the plate, incubation conditions, accurate inoculum density, and the disc must be placed firmly in contact with agar surface (Sandle 2016).

**Penicillin**

Penicillin is a group of antibiotics which includes penicillin G, amoxicillin, and oxacillin. Although penicillins are the longest known beta-lactam antibiotics, this class of compounds still finds widespread application against infectious diseases. Amoxicillin is a semi-synthetic penicillin, moderate–spectrum, bacteriolytic, lactam antibiotic used to treat bacterial infections caused by microorganism which are susceptible to beta-lactamases. Amoxicillin is a bactericide and acts against both Gram–positive and Gram–negative microorganism. It does so
by inhibiting the biosynthesis and repair of the bacterial mucopeptide wall during bacterial multiplication that leads to bacterial death. Amoxicillin is usually combined with clavunic acid which is a β–lactamase inhibitor because amoxicillin is susceptible to degradation by β–lactamase-producing bacteria. This increases effectiveness by reducing its susceptibility to β–lactamase resistance (Ul-Haq and Madura 2016).

Penicillin G or benzylpenicillin is used to treat many bacterial infections and has great activity against staphylococci, streptococci, neisseriae, spirochaetes and certain other organisms. However, due to resistance, normally due to production of β–lactamase, its activity against staphylococci has been undermined. Penicillin G (benzylpenicillin) is rarely used orally because of its poor gastric absorption rate, so it is usually given by injection (Dowd et al. 2017).

Oxacillin is a semisynthetic penicillinase–resistant and acid–stable penicillin. It binds to penicillin–binding proteins in the bacterial cell wall, thereby blocking the synthesis of peptidoglycan, leading to inhibition of cell growth and causes cell lysis. Resistance to oxacillin is common especially among enteric Gram–negative bacilli. However, staphylococci are susceptible because oxacillin is resistant to the bacterial beta–lactamase produced by Staphylococci. It isn’t used therapeutically, but instead used as a marker to test for mec–A–mediated resistance of PBP2a in Staphylococcus sp. (Papich 2016).

**Glycopeptides**

A glycopeptide antibiotic is composed of glycosylated cyclic or polycyclic nonribosomal peptides. It acts primarily by inhibiting cell wall synthesis of bacteria. Glycopeptides are considered antibiotic of a last resort for life threatening infections caused by Gram–positive bacteria which are unresponsive to other less toxic bacteria. This is because glycopeptides are poorly absorbed, not metabolized, excreted renally and are potentially nephrotoxic and ototoxic (Percival et al. 2014). An example of glycopeptide antibiotic is vancomycin. Vancomycin is used for nosocomial infections by methicillin–resistant staphylococci species. Normally, it is administered intravenously to minimize infusion–related side effects (Alt 2018).

**Macrolides**

Macrolide antibiotics contain a large macrocyclic lactone ring to which one or more deoxysugar may be attached. It acts by inhibiting bacterial protein biosynthesis by binding reversibly to the subunit 50s of the bacterial ribosome and preventing translocation of peptidyl–tRNA. Macrolides are commonly used for infections of the upper and lower respiratory tract, and skin and soft tissue infections (Leung 2016). However, the widespread use of macrolides has led to the emergence of macrolide–resistant strains especially among *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. Thus, third–generation macrolides are being developed (Taylor and Triggle 2007). Examples of macrolides are erythromycin, azithromycin, and clarithromycin.
Aminoglycosides

Aminoglycosides are potent, broad spectrum, natural or synthetic antibiotics derived from actinomycetes. They are effective against various Gram-positive and Gram-negative organisms, and are especially potent against bacteria of the Enterobacteriaceae family. It is also active against methicillin-resistant and vancomycin-resistant *Staphylococcus aureus* (Krause et al. 2016). They are used for serious infections caused by Gram-negative bacteria. The disadvantages of aminoglycosides are their nephrotoxic and ototoxic side effects. However, they are still frequently used in underdeveloped countries due to its availability and low cost (Hof and Mobbs 2001). Examples of aminoglycosides are gentamicin, streptomycin, tobramycin and kanamycin.

Polypeptides

Bacitracin is a polypeptide antibiotic that is derived from *Bacillus subtilis*. There are 3 bacitracin subgroups, namely: A, B, and C, with subgroup A being the major constituent of commercial preparations. It contains a thiazolide ring and peptide side ring. It acts by interfering with the dephosphorylation of the lipid compound that carries peptidoglycans to the growing microbial cell wall, thus blocking cell wall formation. It is effective against Gram-positive bacteria including *Staphylococci*, streptococci, *Corynebacterium* sp., and *Clostridium* sp. Resistant in *Staphylococcus aureus* is rare, but has been reported. Bacitracin can’t be use parenterally because it is too toxic, but is well tolerated topically (Dowd et al. 2017).

METHODOLOGY

Time and Place of Research

This study was carried out from the month of September 2018 until November 2018. The samples for bacteria culture were collected from *Purnomo Luak Kampus* Petshop. The identification of the bacteria and the agar disc diffusion tests were carried out at Microbiology Research Laboratory, Medical Microbiology Division, Department of Infectious Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University.

Apparatus and Materials

The apparatus used in this study were glass slides, reaction tubes, petri dish, glass slides, Durham tubes, Eppendorf tubes, gas burner, metal ruler, microscope, micropipette, forceps, inoculation loop, microscope, vortex mixer, 37°C incubator, and refrigerator.
The materials used in this study were Mueller–Hinton agar (MHA), MacConkey agar (MCA), mannitol salt agar (MSA), trypticase soy agar (TSA), blood agar, micropipette tips, sterile water, sodium chloride (NaCl) 0.9% solution, sterile cotton swabs, amoxicillin disc, gentamicin disc, erythromycin disc, bacitracin disc, penicillin G disc, vancomycin disc, oxacillin disc, glucose solution, lactose solution, maltose solution, sucrose solution, trehalose solution, xylose solution, raffinose solution, mannose solution, urease solution, 5% alpha–naphthol, 40% potassium hydroxide, rabbit plasma, hydrogen peroxide (H₂O₂), crystal violet, safrañin, and Lugol’s iodine.

Research Procedure

Collection of Bacterial Samples

Bacterial samples were swabbed from the oral cavity of 5 red–tailed racers (Gonyosoma oxycephalum) in Purnomo Luak Kampus petshop. When more information was enquired regarding the snakes, it was informed that the snakes were wild caught. Swabbed samples were kept in a refrigerator at the Microbiology Research Laboratory of the Faculty of Veterinary Medicine, IPB University.

Identification of Bacteria

Each bacteria sample were streaked onto blood agar and MCA. Twenty-four hours later, multiple different colonies were isolated from the agars, and cultured in slant TSA. Gram staining was carried out to identify Gram-positive bacteria. Test such as catalase test, coagulase test, carbohydrate fermentation test, Voges–Proskauer test, and mannitol salt agar test were carried out to identify the bacteria isolated. The tests were done using 18–24 hours old bacteria.

Preparation of Bacterial Isolates

The bacterial samples were freshly inoculated into slanted agar 24 hours before the study and incubated at 37°C. The bacteria were then made into suspensions equivalent to 0.5 McFarland standard no. 1 which is 1.5 x 10⁸ cfu/mL, by adding the bacteria into sterile NaCl 0.9% solution using an inoculation needle.

Agar Disc Diffusion Method

The antimicrobial susceptibility of the bacterial samples was evaluated with 7 different antibiotics (amoxicillin, gentamicin, oxacillin, penicillin G, bacitracin, vancomycin, and erythromycin) using the agar disc diffusion method. Sterile plates of Mueller–Hinton agar (MHA) were prepared. The plates were swabbed over the entire surface of the agar with a sterile cotton swab dipped in the bacterial suspension of 10⁶ cfu/mL and allowed to dry. For each bacterial sample, 3
repetitions were made by swabbing onto 3 plates. For each plate, antibiotic disc of amoxicillin, gentamicin, oxacillin, penicillin G, bacitracin, vancomycin, and erythromycin were placed on the surface of the agar with sterile forceps. The plates were then incubated at 37°C for 24 hours. After 24 hours, the diameter of the inhibition zones for each antibiotic disc were measured using a metal ruler. The diameter is then compared with Clinical Laboratory Standards Institute 2013 for Staphylococcus spp.

Table 1 Standard diameter of inhibition zones for different antibiotics used against Staphylococcus aureus (CLSI 2013).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Potency</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>20/10 μg</td>
<td>≥ 20</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 μg</td>
<td>≥ 15</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 μg</td>
<td>≥ 23</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>30 μg</td>
<td>≥ 13</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10 μg</td>
<td>≥ 29</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30 μg</td>
<td>≥ 15</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>1 μg</td>
<td>≥ 13</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Identification of Bacteria

Staphylococci bacteria were chosen based on Gram staining test. Staphylococci bacteria are Gram-positive, coccus in shape, and can grow in clusters, pairs, or short chains (Baron 1996). When coagulase test was carried out, no coagulase positive staphylococci were identified. This indicates that the Staphylococci bacteria isolated from the oral cavity of the red–tailed racer are coagulase negative staphylococci (CoNS) species. Further biochemical tests were carried out on the isolated CoNS to differentiate the CoNS species. Table 2 shows the results obtained from the biochemical tests, which were then compared with the table for identification of coagulase negative staphylococci based on Barrow and Feltham (2003). Sample 1 and Sample 6, were isolated from red–tailed racer 1, Sample 2 and Sample 3, were isolated from red–tailed racer 2, Sample 4 and Sample 5 were isolated from red–tailed racer 3, Sample 7 was isolated from red–tailed racer 4, and sample 8 was isolated from red–tailed racer 5.

S1 and S2 are most likely *Staphylococcus xylosus* as they were coagulase negative, catalase positive, VP negative, and fermented lactose, maltose, mannitol, sucrose, trehalose, xylose and mannose, but not raffinose. However, 85-100% of *S. xylosus* strains are usually urease positive. S3 is also *S. xylosus* as it was coagulase negative, catalase positive, urease positive, VP negative, and fermented lactose, maltose, mannitol, sucrose, trehalose, xylose and mannose, but not raffinose. *S. xylosus* is often found as a transient on the skin of lower primates, other mammals and occasionally from birds. It is a common inhabitant of the rodent skin (Gozalo
et al. 2010). It is ubiquitous, and can persist in soils and on surfaces. *S. xylosus* is generally non-pathogenic but a few strains are related to animal and human opportunistic infections (Dordet–Frisoni et al. 2007).

Table 2 Results of biochemical test carried out on the samples

<table>
<thead>
<tr>
<th>Test</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ML</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MN</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>U</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3 Identification of coagulase negative staphylococci based on Barrow and Feltham (2003)

<table>
<thead>
<tr>
<th>Test</th>
<th><em>S. xylosus</em></th>
<th><em>S. sciuri</em></th>
<th><em>S. lentus</em></th>
<th><em>S. kloosii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L</td>
<td>+</td>
<td>-</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>ML</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>S</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>X</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MN</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>U</td>
<td>+</td>
<td>-</td>
<td>d</td>
<td>-</td>
</tr>
<tr>
<td>VP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: C (Catalase test), CG (Coagulase test), MS (Mannitol salt agar), L (Lactose test), ML (Maltose test), S (Sucrose test), T (Trehalose test), X (Xylose test), R (Raffinose test), MN (Mannose test), U (Urease test), VP (Voges - Proskauer test), B (identified bacteria), + (positive), - (negative)

S4, S5 and S6 were identified to be *Staphylococcus sciuri*. For S4 and S5, they were catalase positive, coagulase negative, VP negative, urease negative, and fermented mannitol, maltose, sucrose, trehalose, mannose, but not xylose, lactose and raffinose. For S6, it was catalase positive, coagulase negative, VP negative, urease negative, and fermented mannitol, maltose, sucrose, and trehalose, but not xylose, mannose, lactose and raffinose. Only 16-84% of *S. sciuri* strains will ferment mannose. *S. sciuri* are widely distributed in nature, and they can be isolated
from a variety of animals and the products of animal origin as well as from human. They are usually are apathogenic to animals. However, in Hebei, China, *S. sciuri* was isolated from the pericardial fluid of a diseased piglet with exudative epidermitis. *S. sciuri* is also a human pathogen that is responsible for endocarditis, peritonitis, septic shock, urinary tract infection, pelvic inflammatory disease and wound infections (Chen et al. 2007).

*S7* is *Staphylococcus lentus* as it was catalase positive, coagulase negative, urease negative, and fermented mannitol, maltose, sucrose, trehalose, and mannose, but not lactose, raffinose and trehalose. Usually, 85-100% of *S. lentus* strains are VP positive. *S. lentus* is a commensal bacterium that colonises the skin of several animal species. There have been reported cases of people that work in close contact with animals, being carriers of *S. lentus*. In rare cases, *S. lentus* has caused infection in humans. It has the ability to develop resistance towards macrolide, lincosamide, and streptogramin antibiotics (Schwendener and Perreten 2012).

Lastly, S8 is *Staphylococcus kloosii* because it was catalase positive, coagulase negative, VP negative, and fermented mannitol, lactose, maltose, and trehalose, but not sucrose, xylose, raffinose and mannose. It was urease positive, but 85%-100% of *S. kloosii* strains are usually urease negative. *S. kloosii* has been isolated from various wild mammals including marsupials, rodents and carnivores, but is not known to be a human pathogen. Its colonies can be pigmented or non–pigmented (Kamarudin et al. 2013). In India, there has been a reported case of linezolid resistant *S. kloosii* that was isolated from 60–year–old male with an intracranial bleed and sepsis (Peer et al. 2011).

The results obtained show that red–tailed racer 1 had *S. sciuri* and *S. xylosus*; red–tailed racer 2 had *S. xylosus*; red–tailed racer 3 had *S. sciuri*; red–tailed racer 4 had *S. lentus*; and red–tailed racer 5 had *S. kloosii*.

**Antimicrobial Susceptibility of the Bacteria**

After the bacteria were identified, disc diffusion test was carried out. Table 4 shows the average diameter of inhibition zone of CoNS towards different antibiotics, while table 5 shows the interpreted results of CoNS towards different antibiotics. The results obtained from table 4 is interpreted with the Clinical and Laboratory Standards Institute (2013) for different antibiotics used against *Staphylococcus spp*.

![Figure 1 Kirby-Bauer agar disc diffusion test.](image)
Table 4 Average diameter of inhibition zone of coagulase negative staphylococci towards different antibiotics

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML</td>
</tr>
<tr>
<td>S1</td>
<td>27.6</td>
</tr>
<tr>
<td>S2</td>
<td>25.7</td>
</tr>
<tr>
<td>S3</td>
<td>28.0</td>
</tr>
<tr>
<td>S4</td>
<td>27.7</td>
</tr>
<tr>
<td>S5</td>
<td>23.7</td>
</tr>
<tr>
<td>S6</td>
<td>28.7</td>
</tr>
<tr>
<td>S7</td>
<td>27.3</td>
</tr>
<tr>
<td>S8</td>
<td>33.0</td>
</tr>
</tbody>
</table>

Key: S1-S8 (Sample number), AML (Amoxicillin), CN (Gentamicin), E (Erythromycin), B (Bacitracin), P (Penicillin G), VC (Vancomycin), OX (Oxacillin)

Table 5 Antimicrobial susceptibility of the coagulase negative staphylococci towards different antibiotics

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antimicrobial susceptibility towards the antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML</td>
</tr>
<tr>
<td>S1</td>
<td>S</td>
</tr>
<tr>
<td>S2</td>
<td>S</td>
</tr>
<tr>
<td>S3</td>
<td>S</td>
</tr>
<tr>
<td>S4</td>
<td>S</td>
</tr>
<tr>
<td>S5</td>
<td>S</td>
</tr>
<tr>
<td>S6</td>
<td>S</td>
</tr>
<tr>
<td>S7</td>
<td>S</td>
</tr>
<tr>
<td>S8</td>
<td>S</td>
</tr>
</tbody>
</table>

Key: S1-S8 (Sample number), AML (Amoxicillin), CN (Gentamicin), E (Erythromycin), B (Bacitracin), P (Penicillin G), VC (Vancomycin), OX (Oxacillin), S (Susceptible), I (Intermediate), R (Resistant)

It can be seen that all the CoNS isolated were susceptible to amoxicillin, gentamicin, bacitracin, vancomycin, and oxacillin. This may be because the CoNS were isolated from snakes that have not been in contact with prey or environment containing amoxicillin, gentamicin, bacitracin, vancomycin, and oxacillin, nor have they been in contact with bacteria resistant to the antibiotics stated. This result can be compared to bacteria obtained from farm and domestic animal. Most bacteria from farm and domestic animals are resistant to many antibiotics. For example, a study shows that coagulase positive and coagulase negative Staphylococci isolated from chicken meat are resistant to methicillin, quinolones, beta–lactams, macrolides, lincosamides and streptogamin (Osman et al. 2016). Meanwhile, S. aureus isolated from a pig farm are resistant to tetracycline and macrolides (Oppliger 2012).

The S. xylosus, S. sciuri and S. lentus that was isolated are susceptible to erythromycin, but S. kloosii is resistant to erythromycin. The resistance of S. kloosii towards erythromycin may be due to several factors. For example, red–tailed racer S, in which erythromycin resistant S. kloosii was isolated from, could have eaten prey containing erythromycin resistant bacteria, or drank water containing waste from farms or hospitals that have erythromycin resistant bacteria. Erythromycin is a macrolide. Bacterial resistance to macrolides is often due to efflux systems,
methylases or inactivating enzymes. It can also be caused by mutations in genes encoding ribosomal proteins and in the 23S rRNA gene. These chromosomal mutations alter the erythromycin binding site in the 23S rRNA molecule (Waśko et al. 2012). In S. aureus, erythromycin resistance is usually due either to ribosomal modification by 23S rRNA methylases mediated primarily by \textit{ermA}, \textit{ermB}, or \textit{ermC} or to active efflux of the antimicrobial agent by an ATP–dependent pump mediated by \textit{msrA} (Nicola et al. 1998).

All the CoNS isolated were resistant to penicillin G. Penicillin G is one of the most widely used antibiotic, and is one of the oldest. Many known bacteria have already developed resistant towards Penicillin G. The most important mechanism of resistance to the penicillins is antibiotic hydrolysis mediated by the bacterial enzyme beta-lactamase (Dever and Dermody 1991). Penicillin resistant \textit{Staphylococcus sp.} was documented as early as 1942 (Lobanovska and Pilla 2017). Penicillin G resistant bacteria may have already been present in the environment, leading to the snakes having penicillin G resistant CoNS.

There is a potential for CoNS from animals to emerge as a zoonotic agent. For example, \textit{S. lugdunensis} typically has been associated with human disease, but recently also has been described as an animal pathogen. Meanwhile, \textit{S. schleiferi}, which may be coagulase negative (subsp. \textit{schleiferi}) or coagulase positive (subsp. \textit{coagulans}), typically has been associated with skin infections in dogs and cats, but recently has been described as a human pathogen. Thus, even though CoNS have typically been considered to be less pathogenic than coagulase positive staphylococci, it is important that more concern and studies be done on CoNS. Laboratory methods, especially those based on phenotypic characteristics, may fail to differentiate certain species from each other and from \textit{S. aureus}, and at least one mistake in identification has been reported. While high–risk samples from people, such as blood cultures, may be screened for CoNS, the same attention may not be given to samples from diseased skin (Davis et al. 2013). It is essential to monitor and recognise the changing antimicrobial susceptibility and virulence factors of CoNS that infect both animals and humans.

CONCLUSION AND SUGGESTIONS

Conclusion

The results of this study showed that all 7 samples of \textit{Staphylococcus xylosus}, \textit{Staphylococcus sciuri}, and \textit{Staphylococcus lentus} were susceptible to amoxicillin, gentamicin, bacitracin, erythromycin, vancomycin, and oxacillin, with the exception of one sample of \textit{Staphylococcus sciuri} which was intermediate towards erythromycin. All 6 samples of \textit{Staphylococcus xylosus}, \textit{Staphylococcus sciuri}, and \textit{Staphylococcus lentus} were resistant towards penicillin G. The one sample of \textit{Staphylococcus kloosii} isolated was susceptible to amoxicillin, gentamicin, bacitracin, penicillin G, vancomycin, and oxacillin, but was resistant towards erythromycin.
Suggestions

My suggestion for this research would be to carry out further specific test to better identify the species of bacteria isolated. Further research should also be carried out at the location where the snakes were caught to study how the location and environmental condition effects the bacterial flora of the snakes and their antimicrobial susceptibility. Lastly, the experiment should be repeated using different methods such as the antimicrobial gradient method.

REFERENCES


Bart S. 1996. Medical Microbiology. 4th ed. Galveston (USA): University of Texas Medical Branch at Galveston.


Davis MF, Cain CL, Brazil AM, Rankin SC. 2013. Two coagulase-negative staphylococci emerging as potential zoonotic pathogens: wolves in sheep’s clothing? Front Microbiol. 4: 123


BIOGRAPHY

The author was born in Selangor, Malaysia on the 17th October 1995 to parents Pow Chee Keong and Lee Mooi Yoke. The author is the eldest daughter of two siblings. The author graduated high school in 2012 from Sekolah Menengah Kebangsaan (SMK) Damansara Jaya. In 2015, The author was accepted into the Faculty of Veterinary Medicine at IPB University. The author is a member of the Himpunan Profesi Hewan Kesayangan dan Satwa Akuatik Eksotik.