Science Fair Logbook - Eden

**Is the new antibiotic Darobactin an effective treatment for methicillin-resistant staphylococcus pseudintermedius (and the bacteria it causes) in dogs?**

**(more extensive research on research document; the research on here is only part of it!!)**

Nov 12th

* Started to do background research on the basics
  + Mainly on ‘what is MRSP’

Some Research:

* Methicillin resistant staphylococcus pseudintermedius
  + Staphylococcus pseudintermedius:
    - * Commonly found on the skin, mouth, nose, or gastrointestinal tract
      * Studies suggest that ~50% of dogs are *colonized* with S. pseudintermedius
        + An additional 6-9% are colonized with MRSP
        + Infections are most likely to occur in previously colonized dogs and infected patients probably pose a higher transmission risk than colonized patients
      * Usually does not cause many problems, but opens up opportunities for future infections, and sometimes causes infections right after contracting the bacteria
        + If the pet contracts an illness or gets sick/hurt for another reason, MRSP takes advantage of the body’s weakened defenses, and causes infections/other issues

Also, if the pet is immunocompromised, or has a compromised immune system, it can affect them more severely

* + - * + It commonly causes skin/ear infections, especially if the skin has been damaged (i.e. scratched or affected by allergies)
  + MRSP is just like the bacteria mentioned before, except it is genetically modified to actually be resistant to majority of antibiotics, making it more dangerous
    - researching Darobactin, a new antibiotic found to cure most antibiotic resistant/gram-negative bacteria

Nov 24th

* Continued on more (basic) research

Some Research:

* This bacteria is not well adapted to cause disease in people, rather better adapted to animals, mainly dogs
  + Although dog owners may be exposed to this bacteria on a regular basis, this is not a major concern
    - harm in humans does not go very deep
  + Infections occur commonly in compromised pets
    - Infections happen from direct contact with the bacteria from an inorganic object or other infected animal(s)
  + **Recurrent antibiotic exposure can lead to resistant bacteria in the pet**
    - Can only be diagnosed by a culture and sensitivity performed by a laboratory
      * MRSP infections do not differ from any other type of Staph infection
        + do not look different from infections caused by MSSP or other staph infections
    - *Culture (culturing bacteria): the process of growing a bacterial / other biological unit in an artificial method*
      * All strains of MRSP are resistant to common antibiotics (beta-lactam antibiotics), such as penicillins (i.e. amoxicillin) and cephalosporins (cephalexin)
      * As a result, the bacteria must be **cultured** to choose the best treatment (as mentioned above)
        + Because different strains might be resistant to other antibiotics as well, it still needs to be tested
    - Oral antibiotics can be useful for treatment, but must be chosen based on culture and sensitivity results
      * Local treatment of the outer infection site is often effective
        + I.e. using an antibacterial shampoo

Normally done together with antibiotic therapy

* + - * With these proper steps of treatment, most MRSP infections can be successfully treated within weeks
    - You cannot prevent exposure of your pets/humans to MRSP
      * It is carried by many healthy animals / people
      * One study found MRSP in 4.5% of healthy dogs and 1.2% of healthy cats
        + More recent studies indicate a **greater percentage of healthy dogs and cats now carry MRSP**

**MRSP infections appear to be increasing substantially in animals (particularly dogs)**

Also very large increases in MRSP skin infections (pyoderma)

Infections after surgery are also becoming more common, and these are more difficult to treat

* + - * + MRSP is an opportunistic bacteria, so the skin must be damaged by something in order for it to cause an infection
        + MRSP (and s. Pseudintermedius in general) is well designed to live on pets

Not much information has been found on this topic yet, but it is possible that the carriage of MRSP can occur for *months*

* + - Non MRSP forms of *s. Pseudintermedius* are methicillin susceptible (not resistant to methicillin)
  + Methicillin : a semisynthetic penicillin-related antibiotic, aka Staphcillin
    - Was once effective against staphylococci bacteria resistant to penicillin
      * Now rarely used, has been largely superseded by Vancomycin
        + Vancomycin : antibiotic used to treat a number of bacterial infections

Recommended as a treatment for complicated skin, bloodstream, endocarditis, bone and joint infections, and meningitis cause by MRSA (methicillin resistant Staphylococcus aureus

* + MRSP vs MRSA
    - MRSP : methicillin resistant staphylococcus pseudintermedius
    - MRSA : methicillin resistant staphylococcus aureus
      * MRSA is the antibiotic-resistant form of S. aureus
    - *In people, MRSA is a huge problem, but MRSP in people is only a minor concern*
    - *In dogs, MRSP is a major health problem, but MRSA infections are much less common*
      * MRSA can infect both people and animals, and can be transmitted between people and pets relatively easily, in both directions
        + MRSP can also be transmitted from pets to people, but this is uncommon

The zoonotic disease risk from MRSP in animals *is low* (as already mentioned above in notes)

* + What can MRSP cause?
    - In dogs/cats, MRSP most commonly causes skin and ear infections
      * Wound infections, surgical site infections, and other types of infections can also occur, although the most common is a skin infection
        + Its can also cause severe diseases such as ‘flesh eating disease’

But this is very rare

More severe cases also result is MRSP is not diagnosed quickly and ineffective treatment (such as antibiotics that MRSP is resistant to) are used

Nov 26th

* Continued researching basics again
  + Summarizing research on MRSP and similar bacteria
* Created hypothesis
  + Hypothesis : If the new antibiotic Darobactin is used to treat MRSP in dogs, then it would have a good effect on the stoppage of the bacteria (and the bacteria that MRSP could cause) overall because Darobactin is able to treat many kinds of antibiotic resistant or gram-negative bacteria, and MRSP itself is gram-negative or antibiotic resistant.

Research:

* + Studies suggest that ~50% of dogs are *colonized* with S. pseudintermedius
    - An additional 6-9% are colonized with MRSP
    - Infections are most likely to occur in previously colonized dogs and infected patients probably pose a higher transmission risk than colonized patients
    - If your dog has MRSP, you should not put yourself in contact with the dog either
  + You can prevent MRSP from overall happening a little bit by using antibiotics responsibly yourself and for your pets
  + Colonized animals : animals/people that develop MRSP without symptoms (asymptomatic people)
    - There is no info about when/how to treat MRSP in colonized animals
      * S. pseudintermedius evolved to live on dogs and cats, so decolonizing these animals may be very difficult or even impossible
        + Decolonization therapy with antibiotics in *not* recommended for animals colonized with MRSP

Unlikely to be effective and could lead to more bacterial antimicrobial resistance in the future

* + - * MRSP can occur very frequently if people do not wash their hands after contact with an infected animal/person
      * Hospitalized MRSP patients must be separated to manage contact transmission
      * If discharge cannot be contained (i.e. draining wounds cannot be bandaged), patients must be housed in isolation
      * Hand washing or use of hand sanitizers must be enforced after any contact with affected patients
      * There is negligible zoonotic hazard for personnel related to MRSP exposures
  + Antibiotic resistance in dogs
    - Occurs when disease-causing bacteria develop the ability to resist some antibiotics
      * Currently increasing in certain bacteria that are important in canine health
        + Leads to infections that are difficult/impossible to treat

+ extended hospital stays and additional follow-up visits

Costly alternatives - may have negative side effects

Dec 11th

* Continuing background research - Darobactin basics

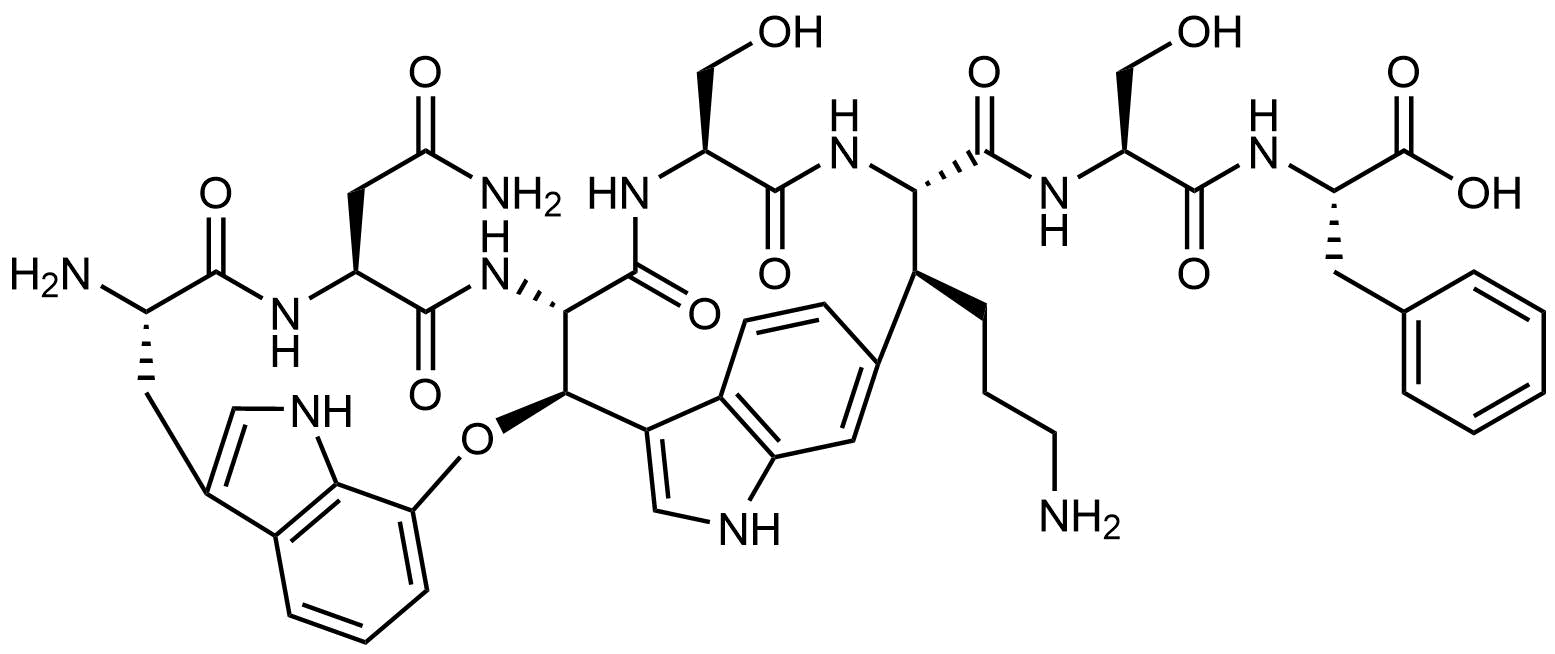
Research:

* Discovery of Darobactin
  + first discovered by Professor Till Schaberle
    - At the Institute of Insecticidal Biotechnology
  + Being further researched also by Dr. Kim Lewis from the National Institute of Allergy and Infectious Diseases (NIAID)
  + Discovered on November 21, 2019 at Institute of Biotechnology
    - Found to find a possible cure for antibiotic-resistant bacteria
    - achieved because scientists found a new chemical inside of a new species of insect that could help battle bacteria

Dec 15th

* More research on Darobactin and beginning to research gram negative/positive bacteria

Research:

* Darobactin can cure Gram-negative bacterias that are resistant to common antibiotics
  + 
    - * ^^^ chemical components of Darobactin
* **Gram’s method of staining / Gram positive and Gram negative bacteria**
  + Gram’s Method of Staining - way to classify bacteria
    - The Gram Test is staining the cell walls of the separate bacterias
      * common stains used are fuchsine or safranin, chemicals that have heavy dye levels
      * crystal violet is used as a main stain, others like safranin or fuschine used as counterstains
      * two (main) classifications; Gram-positive and Gram-negative
        + Also Gram-variable bacteria, acid fast bacteria and atypical bacteria
    - Gram-positive
      * Bacteria that stay the stained colour because of a thicker cell wall, meaning that the wall clings on to the colour more, even for the decolourization
      * More peptidoglycan
        + Peptidoglycan is a particular layer in the cell walls of the bacteria

the place where action of beta-lactam antibiotics such as penicillins and cephalosporins do their job

* + - * This bacteria is easier to defeat because of its cell wall because the antibodies could stick to the outer wall, rather than just slide off
      * An example of a Gram-positive bacteria would be bacterial pneumonia

Dec 18th

* Continuation on gram negative/positive bacteria
  + Focus on gram-negative

Research:

* Gram-negative
  + Gram-negative bacteria are bacteria that reacts differently to the stain and turns a different colour than the used stain
    - The cell wall of these bacteria is thinner
      * Colour normally wipes out when decolourized and counterstained
      * Less peptidoglycan
        + Thinner layer that does not retain crystal violet, so when safranin is added, gram-negative bacteria stain red
        + Harder to combat because there is not as much peptidoglycan, leaving less space for the antibiotics to do their job
    - An example of a Gram-negative bacteria would be bacterial meningitis

Dec 20th

* Research on outlier bacteria - focus on gram-variable
  + Gram-variable bacteria
    - Organisms that take up the positive stain variably
    - Organisms such as clostridium species will be gram-variable, or even appear gram-negative on smears directly from patient specimens
      * Clostridium species : a genus of gram-positive bacteria belonging to the phylum Firmicutes
      * When these organisms are grown in the lab and then stained, they are strongly gram-positive
    - There are rare circumstances of gram-negative bacteria such as Moraxella and Acinetobacter that tend to appear to be gram-positive
    - Use of the term ‘gram-variable’ restricted to cases where the organism cannot be determined as gram-positive or negative
      * Sometimes, debris and artifacts can imitate bacteria/organisms
        + Making it seem ‘gram-variable’
    - Some bacteria, when stained, show a mix of purple+pink cells
      * Also gram-variable bacteria

Dec 23rd

* Research on outlier bacteria - focus on acid-fast bacteria

Research

* Acid fast bacteria / acid fast bacillus
  + Sometimes called AFB
    - Acid fast bacteria is a group of bacteria with the characteristic of acid fastness
      * Acid fastness: a physical property that allows a bacterium to resist decolorization by acids during staining procedures (such as Gram’s Method of Staining)
        + So once the bacterium is stained, it cannot be decolored using acids (as routinely done in the process)

Acid fast bacteria can be classified and detected with quite simple laboratory procedures (i.e. microscopy) because of their unique features

* + - * Examples of acid fast bacteria:
        + Genus Mycobacterium – M. leprae, M. Tuberculosis, M. smegmatis, M. Avium complex, M. kansasii.

Mycobacterium tuberculosis - more commonly known

* + - * + Genus Nocardia – N. brasiliensis, N. cyriacigeorgica, N. farcinica, and N. nova.

Nocardia farcinica - more commonly known

Dec 27th

* Research on outlier bacteria - focus on atypical bacteria

Research

* Atypical bacteria
  + Bacteria that do not colour with gram-staining
    - Remain colourless
      * Not gram positive or negative
    - Examples :
      * Chlamydiaceae, legionella, mycoplasmataceae, rickettsiaceae, spirochetes (i.e. leptospirosis)
        + Chlamydiaceae and mycoplasmataceae do not have a peptidoglycan layer

Do not retain crystal violet or safranin, resulting in no colour

Peptidoglycans are where the action of antibiotics takes place, so chlamydia and mycoplasma are naturally resistant to these

Making them antibiotic resistant

* + - * + Rickettsiaceae are technically Gram-negative, but too small to stain well

Often considered atypical

* + Macrolides such as erythromycin are usually effective in treating atypical bacterial infections.
  + some of these bacteria can cause a specific type of pneumonia referred to as atypical pneumonia
    - atypical pneumonia is not only caused by atypical bacteria
      * this disease can also have a fungal, protozoan or viral cause

Dec 29th

* Did basic research on Staphylococcus intermedius
  + Related to S. pseudintermedius
    - Wanted to research to see the connection between S. pseudintermedius and S. intermedius and how it played a role

Research

* Staph. intermedius (in dogs)
  + Staphylococcus intermedius
    - Part of the normal skin and oral flora of dogs and a variety of other animals (cats, pigeons, minks, horses, foxes, raccoons, goats, gray squirrels, etc)
    - Predominant cause of skin/soft tissue infection in dogs
      * Infections of humans rare
      * True incidence is unknown because S. intermedius is frequently misidentified as S. aureus
        + Most reported cases in humans have been related to dog exposure
      * Large range of cases - ranging from soft tissue infections to brain abscess
    - Increasing number of cases with serious invasive infections with S. intermedius in humans
      * Including infected dog bite wounds, bacteremia, pneumonia, sinusitis, otitis externa, nail bed infection, mastoiditis, brain abscess, skin abscess,etc etc
    - One outbreak of S. intermedius related food intoxication
      * Involving over 265 cases in the western US in 1991
    - Discovery of S. pseudintermedius led to the reclassification of isolates previously identified as s. Intermedius based on molecular techniques
      * According to this grouping, S. pseudintermedius, not S. intermedius, is the bacillum that colonizes and causes infections in dogs/cats
    - S. intermedius mainly colonizes pigeons
      * Older reports of S. intermedius, particularly in animal bites, are often now seen as S. pseudintermedius cases
  + MRSI (methicillin-resistant staphylococcus intermedius) has emerged, and should be a concern along with MRSP

Jan 6th 2021

* More research on darobactin - more specific

Jan 7th 2021

* Reading specific articles and studies related to antibiotic resistance and discovery

Jan 10th 2021

* Began writing email templates - first draft below

Jan 14th 2021

* Found three experts to email
* Finished first template

Emails:

[info@westmountvet.ca](mailto:info@westmountvet.ca)

[signalhillanimalclinic@gmail.com](mailto:signalhillanimalclinic@gmail.com)

[gac@glamorgananimalclinic.com](mailto:gac@glamorgananimalclinic.com)

Jan 15th 2021

* Found four more experts
* Sent out all expert emails

Emails :

[k.lewis@northeastern.edu](mailto:k.lewis@northeastern.edu)

[ebrown@mcmaster.ca](mailto:ebrown@mcmaster.ca)

[Till.F.Schaeberle@agrar.uni-giessen.de](mailto:Till.F.Schaeberle@agrar.uni-giessen.de)

[tschneider@uni-bonn.de](mailto:tschneider@uni-bonn.de)

Jan 16th

* Two expert email replies
  + From:
    - Dr. Eric Brown
    - Dr. Till Schaeberle
      * 2/7 expert replies
  + Noted info/research gathered from emails
  + Started working on powerpoint

Jan 17th

* Two expert email replies
  + From:
    - Dr. Kevin MacAulay - Glamorgan Animal Clinic
    - Dr. Timsy Bhando (referred/contacted by another expert, Dr. Eric Brown)
* Continued working on powerpoint

Jan 19th

* Replied to Dr. Schaeberle
* Continued working on powerpoint

Reply :

Hi Dr. Schaeberle,

First of all, thank you for replying to my questions. The information you provided to me was very helpful to my project overall.

Secondly, I apologize for asking about staphylococci in general, I meant to ask about another bacteria. I understand that usual staphylococci is gram-positive, but I was actually meaning to ask about gram negative bacteria that staphylococci can cause, such as bacteria causing infections. A more appropriate question would be: Could you give me a general picture of what strains of bacteria Darobactin has been tested on or will soon be tested on, and their effectiveness so far?

Thanks,

Eden

Jan 20th

* Received reply from Dr. Schaeberle
* Finalized conclusion
* Continued working on powerpoint

Conclusion:

My hypothesis was correct because after analyzing all of the data brought to me, I concluded that the antibiotic Darobactin would be suitable for treating MRSP and most infections it causes. Darobactin, from the research I collected, would be able to battle MRSP and also many of the gram-negative infections it can cause. The new antibiotic has already been tested on many bacteria and found to be positive, and most of these bacteria are all similar or related to MRSP and the bacteria it can cause in some way. There are also no limiting factors saying that Darobactin will not be effective in dogs, as most antibiotics today are effective in both humans and our household pets and I have found no evidence suggesting Darobactin will be any different.

Jan 21st

* Continued working on powerpoint

Jan 22nd

* Continued working on powerpoint
* Making notes on information received from Dr. Bhando

Jan 23rd

* Continued working on powerpoint
* Gathering sources used

Jan 24th

* Continued working on powerpoint
  + Finalizing overall presentation

Jan 25th

* Continued working on powerpoint
  + Adjusting final details

Jan 26th

* Continued working on powerpoint
  + Practicing presentation

Jan 27th

* Continued working on powerpoint
  + Practicing presentation

Jan 28th

* Continued working on powerpoint
  + Practicing presentation

Jan 29th

* Presentation day for school science fair

Feb 5th

* Found out that my project is advancing to CYSF

Feb 6th

* Made quick adjustments to slides

Feb 8th

* Continued fixing slides according to feedback

Feb 10th

* More work on project slides and script

Feb 12th

* Continued slideshow/script edits

Feb 14th

* Continued slideshow/script edits

Feb 16th

* Continued slideshow/script edits
* Practiced presentation
* Started research on how darobactin works vs ampicillin

Feb 17th

* Worked on slideshow/script and practiced

Feb 18th

* Worked on slideshow and practiced

Feb 19th

* Did in class presentation

Feb 20, 23, 27

* Edited slideshow
* Began prepping cysf resources (video, banner etc)

March 1-10

* Continued slideshow edits
* Continued cysf resource creation

March 11-15

* Finalized resources

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### ╔══**Expert emails**══╗

#### List of experts emailed:

Replied - ✓ beside name

Not replied - ☐ beside name

☐ [info@westmountvet.ca](mailto:info@westmountvet.ca) - vets at Westmount Vet - (Dr Jeff Dand, Dr Mallory Green, and Dr. Emilia Balc)

☐ [signalhillanimalclinic@gmail.com](mailto:signalhillanimalclinic@gmail.com) - vets at Signal Hill Animal Clinic - Dr. Michael Sweet, Dr. Laurina LeBoldus and Dr. Elizabeth Cumyn,

✓ [gac@glamorgananimalclinic.com](mailto:gac@glamorgananimalclinic.com) - vets at Glamorgan Animal Clinic - Dr. Kevin MacAulay, Dr Amelia Falk, Dr Karen Hill and Dr. Jane Mahon

☐ [k.lewis@northeastern.edu](mailto:k.lewis@northeastern.edu) - Dr. Kim Lewis / North Eastern University

✓ [ebrown@mcmaster.ca](mailto:ebrown@mcmaster.ca) - Dr. Eric Brown / McMaster University

✓ [Till.F.Schaeberle@agrar.uni-giessen.de](mailto:Till.F.Schaeberle@agrar.uni-giessen.de) - Dr. Till Schaeberle / Institute of Insecticidal Biotechnology

☐[tschneider@uni-bonn.de](mailto:tschneider@uni-bonn.de) - Dr. Tanja Schneider / University of Bonn

✓ [bhandot@mcmaster.ca](mailto:bhandot@mcmaster.ca) - Dr. Timsy Bhando / McMaster University (contacted by Dr. Brown)

### First email template (for veterinarians)

Dear [insert name of expert here],

My name is Eden Xu, a grade seven student at Louis Riel School. I am starting my science fair project this year and my topic is about MRSP (methicillin-resistant staphylococcus pseudintermedius) in dogs, relating to the new discovery of the antibiotic Darobactin. My project this year is a research project, so I need to do a lot of extensive research.

Since veterinarians are experts with animals and their health, I was wondering if you could answer some of my questions and help with my research. I emailed your clinic with questions about my last year’s science fair topic as well, and I am grateful that you helped me then and I hope that you can help me again. I have done some research and I know some useful and basic information already about my topic.

Some of the questions I would like to ask you include:

* Can you give me a general picture of how you would treat diseases that are antibiotic resistant in dogs?
* Has Darobactin been tested on any (household) pets
* Is there any information or thoughts you can give on Darobactin treating household pets?
* Have you ever treated any cases of MRSP or Staph. Pseudintermedius? If so, what treatments did you use?
* Do you think that Darobactin would be effective on dogs or other household pets?
* Have any cases of gram-negative bacteria occurred in dogs, and if so, what types were they and how were they treated?
* Do you have any other additional information about MRSP, antibiotic resistance in dogs, or Darobactin (relating to dogs)?

If it is okay with you, you could respond by email if that works for you. If you don’t have time to reply to this email, that is perfectly fine, I understand you are busy. Any help or resources you can provide me with would be greatly appreciated.

Thank you so much for your time in supporting my project.

Sincerely,

Eden

### Second Email template (for general experts) :

Dear [insert name],

My name is Eden Xu, a grade seven student at Louis Riel School in Calgary, Canada. I am starting my science fair project this year and my topic is about MRSP (methicillin-resistant staphylococcus pseudintermedius) in dogs, relating to the new discovery of the antibiotic Darobactin. My project this year is a research project, so I need to do a lot of extensive research.

I was wondering if you could answer some of my questions and help with my research. I have also looked into your discovery of the antibiotic Darobactin, so I understand that you are an expert in this field. I have done some research and I know some useful and basic information already about my topic.

Some of the questions I would like to ask you include:

* Has Darobactin been tested on any strain of staphylococcus bacteria?
* When do you believe that Darobactin will be ready for general use in the population?
* Have you ever seen any cases of MRSP or Staph. Pseudintermedius? If so, could you describe how they were treated and what the case was like?
* Do you think that Darobactin would be effective on dogs or other household pets or animals?
* Do you think that Darobactin could go on to treat a large range of diseases?
* Do you have any other additional information about MRSP, antibiotic resistance, or Darobactin that you think would be useful for me?

If it is okay with you, you could respond by email if that works for you. If you don’t have time to reply to this email, that is perfectly fine, I understand you are busy. Any help or resources you can provide me with would be greatly appreciated.

Thank you so much for your time in supporting my project.

Sincerely,

Eden X.

### Third Email Template(reply)

Hi, [expert name],

Thank you so much for your time and help! Your support has helped me a lot. I will make sure to reference you while presenting my project at science fair.

Thanks again,

Eden X.

### Fourth Email Template(reply2) - referrals to other experts?

Hi, [expert name],

Thank you so much for sending my questions to another expert, and I really appreciate your help in giving me more contacts of people who are specialists in this field.

Thanks again,

Eden X.

### Other custom replies

[replying to Dr. Till Schaeberle’s first email]

Hi Dr. Schaeberle,

First of all, thank you for replying to my questions. The information you provided to me was very helpful to my project overall.

Secondly, I apologize for asking about staphylococci in general, I meant to ask about another bacteria. I understand that usual staphylococci is gram-positive, but I was actually meaning to ask about gram negative bacteria that staphylococci can cause, such as bacteria causing infections. A more appropriate question would be: Could you give me a general picture of what strains of bacteria Darobactin has been tested on or will soon be tested on, and their effectiveness so far?

Thanks,

Eden

#### **Email(s) from Dr. Brown**

1.

Hi Eden,

What a great science project! I have fond memories of my first science fair when I was about your age.

I am copying Dr. Timsy Bhando in my research group who will be delighted to help with your questions.

Best of luck,

Eric Brown, Ph.D.

#### **Email(s) from Dr. Timsy Bhando**

Hi Eden,

Hope you are doing good!

I am sorry for my late response to your questions. Please find the answers in the attached file. I have listed suitable references wherever possible. Do let me know if you are unable to access/download any of these research articles.

Good luck for your project and future endeavors!

Best,

Timsy

Response in attached document:

**Can you give me a general picture of how antibiotic resistant diseases (such as MRSA or MRSP) would be treated?**

Antibiotics have long been the mainstay for antibacterial therapy against infections caused by *Staphylococcus aureus* or*Staphylococcus pseudintermedius.* Vancomycin is the most commonly administered antibiotic for the treatment of MRSA infections, however, the emergence of Vancomycin-intermediate *S. aureus* (VISA) and vancomycin resistant (VRSA) strains limits its use. Daptomycin, ceftaroline, linezolid are a few other antibiotics that inhibit MRSA infections.

Combination therapy is also used to treat MRSA infections such as Combination of a glycopeptide or lipopeptide and a ß-lactam antibiotic such as first- or fourth-generation cephalosporin, or ceftaroline. Combination therapy with daptomycin and ß-lactams has also been reported. The combination of daptomycin with fosfomycin or daptomycin with trimethoprim/sulfamethoxazole (TMP/SMX) have also demonstrated efficacy in clinic.

Several novel antimicrobials have recently been developed and are in various stages of clinical trials, including ceftobiprole, dalbavancin, omadacycline, oritavancin, iclaprim and delafloxacin.

The following papers updates on the treatment options available for *S. aureus* infections.

Turner, N.A., Sharma-Kuinkel, B.K., Maskarinec, S.A. *et al.* Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol* 17**,**203–218 (**2019**).

Lee, A., de Lencastre, H., Garau, J. *et al.* Methicillin-resistant *Staphylococcus aureus*. *Nat Rev Dis Primers* 4**,**18033 (**2018**).

Antivirulence compounds are also promising alternatives that have the potential to attenuate bacterial virulence and reverse antibiotic resistance. A recent study identified anti-virulence compound, MAC-545496 that reversed resistance to various β-lactam antibiotics including penicillins, cephalosporins and imipenem etc. in MRSA.

El-Halfawy, O.M., Czarny, T.L., Flannagan, R.S. *et al.* Discovery of an antivirulence compound that reverses *β*-lactam resistance in MRSA. *Nat Chem Biol* 16,143–149 (**2020**).

Over the past decade, MRSP has also emerged as a clinically important pathogen and resistance to several classes of antimicrobials, such as the fluoroquinolones, macrolides, aminoglycosides and tetracyclines has been reported. Few antimicrobials that exhibit activity against MRSP strains include amikacin, rifampicin, vancomycin and linezolid.

# Triple combination therapy using the combination of antibiotics meropenem/piperacillin/tazobactam, also has the potential to inhibit clinical isolates of *S. aureus* and *S. pseudintermedius.*

# Yoneda A, Thänert R, Burnham CD, Dantas G. In vitro activity of meropenem/piperacillin/tazobactam triple combination therapy against clinical isolates of *Staphylococcus aureus, Staphylococcus epidermidis*, *Staphylococcus pseudintermedius* and vancomycin-resistant *Enterococcus* spp. *Int J Antimicrob Agents*. **2020** Feb

The use of natural products such as manuka honey or essential oilsagainst resistant *Staphylococcus pseudintermedius* strains have also been reported. Bacteriophages and antimicrobial peptides with activity against MRSP have also been discovered.

Antibacterial and Antivirulence Activity of Manuka Honey against Genetically Diverse *Staphylococcus pseudintermedius* Strains. Helen L. Brown, Georgie Metters, Matthew D. Hitchings, Thomas S. Wilkinson, Luis Sousa, Jenna Cooper, Harry Dance, Robert J. Atterbury, Rowena Jenkins. *Applied and Environmental Microbiology,*86(20),Oct **2020**

Nocera, F.P.; Mancini, S.; Najar, B.; Bertelloni, F.; Pistelli, L.; De Filippis, A.; Fiorito, F.; De Martino, L.; Fratini, F. Antimicrobial Activity of Some Essential Oils against Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus pseudintermedius*-Associated Pyoderma in Dogs. *Animals* **2020**

Moodley A, Kot W, Nälgård S, Jakociune D, Neve H, Hansen LH, Guardabassi L, Vogensen FK. Isolation and characterization of bacteriophages active against methicillin-resistant Staphylococcus pseudintermedius. Res Vet Sci. **2019** Feb; 122:81-85.

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**Has Darobactin been tested on any strain of staphylococcus bacteria?**

Darobactin was tested on *Staphylococcus aureus* strainHG003 and itsMinimum Inhibitory Concentration was observed to be >128 µg/ml, while it had a better activity i.e. a lower MIC against Gram negative bacteria such as polymyxin-resistant *Pseudomonas aeruginosa*, β-lactam-resistant *Klebsiella pneumoniae* and *Escherichia coli.*

**When do you believe that Darobactin will be ready for general use in the population?**

It is difficult to say when exactly Darobactin would be available to be used clinically. Prof. Kim Lewis’s group at North Eastern University, USA discovered Darobactin in 2019 (Imai *et al.,* Nature, 2019). Currently the Lewis lab is working on the synthesis of darobactin analogs and optimisation of its activity.

The process of discovering and developing new antibiotics is seemingly complex, time-consuming, and expensive. Moving a potential antibiotic candidate from concept to market may take as long as 15 years and can cost approximately $1 billion. Following early laboratory research for antibacterial discovery, molecules are subjected to preclinical testing which involve extensive laboratory and animal experiments to determine their safety for human testing. Following preclinical testing, a drug undergoes multiple phases of clinical trials that aims to gather evidence for its safety and efficacy, ultimately leading to its approval by FDA for clinical use.

Considering the promising antibacterial activity, safety and efficacy of darobactin as demonstrated by the Lewis lab, I believe it has the potential to enter phase I clinical trials soon.

**Have you ever seen any cases of MRSP or *S. pseudintermedius*? If so, could you describe how they were treated and what the case was like?**

I haven’t come across any case of MRSP or *Staph. pseudintermedius.*

**Do you think that Darobactin would be effective on dogs or other household pets?**

Yes, considering the excellent antibacterial activity of darobactin in a wide variety of pathogens, I do think that it would be effective on household pets. However, since it costs a lot of effort, time, and money to bring an antibiotic from benchside to clinic, only time will tell if darobactin qualifies for it.

**Have any cases of gram-negative bacteria occurred in dogs, and if so, what types were they and how were they treated?**

Yes, members of the Enterobacteriaceae family which includes many Gram-negative species such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. and *Salmonella* spp are known to cause infections in dogs.

The most commonly used antimicrobials for dogs are β-lactams such as amoxicillin, amoxicillin in combination with clavulanic acid, carbapenems; aminoglycosides such as amikacin, or tobramycin and first-generation cephalosporins.

Papich MG. Antibiotic treatment of resistant infections in small animals. Vet Clin North Am Small Anim Pract. 2013 Sep;43(5):1091-107. doi: 10.1016/j.cvsm.2013.04.006. Epub 2013

**Do you have any other additional information about MRSP, antibiotic resistance, or Darobactin?**

Would like to share a list of antibiotics that have been recently approved by FDA for clinical use against bacterial infections. The ones active against Staphylococcal infections are highlighted in the table. (File attached).

The following article from Wellcome trust highlights the difficulties of the process of antibiotic discovery: https://wellcome.org/news/why-is-it-so-hard-develop-new-antibiotics

#### **Email(s) from Dr. Schaeberle**

1.

Dear Eden Xu,

thanks for your interest in the antibiotics field and in antibiotic resistance.

This can be seen as one of the biggest threat to human health (classified like this by the WHO). However, currently we are facing an pandemic situation. However, some people say antibiotic resistance can be seen as a slow pandemic situation. It is happening, there are already pathogens out there for which no effective antibiotic is available.

Darobactin is a compound that is only active against Gram-negative bacteria. Staphylococci are Gram-positive. Please have a look on this. The Gram-negatives have an additional outer membrane that protects them, since most antibiotics cannot reach their target.

It will take many years until Darobactin will be developed into a medicinal drug. Up to now there are no red flags that will stop development; however, this is a very long process.

In general, antibiotics are of course effective for treatment of household pets like dogs. However, like for us it is important to only use antibiotics when they are really needed to not foster resistance development.

I am hoping that Darobactin could become an antibiotic in the future….but as mentioned this is still a long way (about 10 years) to go.

Good luck with your project,

Best wishes,

Till

2.

…attached a slide with the activities tested.

In red the Gram-negative test bacteria and in blue Gram-positive as well as gut symbionts. The cool thing is that Darobactin is not killing the gut symbionts (Bacteroidetes).

Best,

#### **Email(s) from Dr. MacAulay - Glamorgan Animal Clinic**

1.

Hi Eden,

It is great to hear that you have such a strong interest in science, and in particular a topic that is related to veterinary science! I am happy to assist you as best as I can with you questions.

First of all, I have never heard of darobactin until your email! It is that new! So, I had to do a search on the Veterinary Information Network (VIN) that I subscribe to in order to learn more about it, and only one article came up about darobactin. You would need a subscription to the journal *Nature* in order to read the whole submission, but here is the summary that was provided: [summary below in additional articles]

Here are my responses to your list of questions:

* When we have to deal with diseases that are antibiotic resistant in dogs, we try to use a treatment that utilizes a different pathway to kill bacteria than the pathway that the resistant antibiotic would have utilized. For example, many *Pseudomonas* bacterial ear infections in dogs can be difficult to resolve as the *Pseudomonas* can be quite resistant to many antibiotics. However, with the use of a special ear wash solution called TrizEDTA that you flush into the ear about 20-30 minutes prior to the use of an ear antibiotic, the TrizEDTA can change the bacterial wall and make it more permeable or accessible for the antibiotic to now pass through the bacterial wall and into the bacteria to kill it. In addition, sometimes we will have a lab test performed called a culture and sensitivity test to help guide our decision making for treatment. The sample from the dog is sent to the laboratory and is grown on special growth mediums (or cultures) in order to identify the bacteria in question, and then a multitude of various antibiotics are used on the bacteria to see if the antibiotics can stop the growth of the bacteria. If an antibiotic can stop the growth during this testing, we consider the bacteria to be "sensitive" to this antibiotic, and this antibiotic joins a list of possible options to use to treat the dog. If the antibiotic cannot stop the growth during this testing, then we consider the bacteria to be "resistant" to this antibiotic, and try to avoid using this antibiotic in the dog's treatment plan.
* As I mentioned above, I have never heard of darobactin before, and thus I am not aware that it is or has been tested on any household pets. If I were to hazard a guess, I would assume that any new treatment with promise to help fight infections, such as darobactin, would currently be in testing for all kinds of species, including dogs. This type of research is likely ongoing as we speak, and those veterinarians that work in research and development for drug manufacturing companies would know. Veterinarians in clinical practice (such as me) only learn about a new drug once it has been placed through years of thorough research, followed by rigorous testing by health agencies such as Health Canada that need to approve the use of the drug before it can be mass produced, delivered and then administered to the rest of the population.
* I am going to answer your third and fifth questions together here as they are similar and I think best answered together. Based on the scant information available that I could find online that I was able to read about darobactin, my initial thoughts are that darobactin may be a promising and effective treatment option for dogs or other household pets. Anytime researchers find novel pathways for an antibiotic to kill bacteria, then it allows opportunity to overcome the pathways tried before that the bacteria have now become resistant to. More research is needed first and several thoughts come to mind for me as a clinical practitioner such as: is it a safe antibiotic; what side effects could it cause; what is the most effective dose needed for it to work; what is the smallest dose we could use and it would still work; does it work both *in vitro* and *in vivo* situations; does it work in all or only certain species; how long does it last for once it is administered; what form would it come in (i.e. pill, capsule, liquid, topical, injectable), etc.?
* Yes, I have had to treat a few cases of MRSP. In these cases, through the guidance of culture and sensitivity testing as I mentioned above, I was able to choose a different oral antibiotic that the MRSP was sensitive to, and treated the patient that way. In some other cases, the MRSP was a skin infection that was treated not with oral antibiotics, but rather through topical treatment. I used a medicated shampoo and spray that contained a highly concentrated antimicrobial compound called chlorhexidine. Chlorhexidine is the same antimicrobial compound we have in the soap we scrub our hands and arms with before we do surgery in order to eliminate bacteria off our skin surface. The MRSP patients with skin infections were bathed with the mediated shampoo every 3-4 days with the shampoo left on the patient for 10 minutes to have time to kill the bacteria before it was rinsed off. Then, in between the baths, the medicated spray was "spritzed" over the infected skin area and left on so that the antimicrobial properties of the chlorhexidine would be present in between the medicated baths to continue to kill the MRSP. Here are links to the medicated topical products we use, such as Douxo Pyo: <https://www.douxo.ca/> and Pro-Hex: <https://proconceptsanimalhealth.com/products/career-opportunity/prohex/>
* Yes, gram-negative bacterial infections occur in dogs very regularly. We see them every day. These include *Pseudomonas aeruginosa* (as I already mentioned above), as well as *E.coli*, *Klebsiella*, *Proteus*, *Salmonella*, *Enterobacter*, and *Campylobacter*. They were treated with many of the different oral, injectable or topical antibiotics we have, and fortunately most have been effective.
* Unfortunately, I do not have any additional information about darobactin. However, I do have access to lots of information about MRSP and antibiotic resistance in dogs. The question for you is: where do you want to start on this topic as it is so vast! I will include some general info below (including info from my pharmacology professor at the vet school I graduated from in Saskatoon) and email you some others separately. Also, here is a link to some great info as well from our provincial veterinary association; <https://www.albertaanimalhealthsource.ca/topic/antimicrobial-resistance>

Let me know if you need clarification on any of the information I provided above or if any other questions arise, or if you want more info specifically on MRSP and antibiotic resistance.

Good luck!

##### **Additional articles from Dr. MacAulay’s email**

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**A new antibiotic selectively kills Gram-negative pathogens.**

Language: English

Nature. December 2019;576(7787):459-464.

DOI: [10.1038/s41586-019-1791-1](http://dx.doi.org/10.1038/s41586-019-1791-1)

Yu Imai 1, Kirsten J Meyer 1, Akira Iinishi 1, Quentin Favre-Godal 1, Robert Green 1, Sylvie Manuse 1, Mariaelena Caboni 1, Miho Mori 1, Samantha Niles 1, Meghan Ghiglieri 1, Chandrashekhar Honrao 2, Xiaoyu Ma 2, Jason J Guo 2, Alexandros Makriyannis 2, Luis Linares-Otoya 3, Nils Böhringer 3, Zerlina G Wuisan 3, Hundeep Kaur 4, Runrun Wu 5, André Mateus 6, Athanasios Typas 6, Mikhail M Savitski 6, Josh L Espinoza 7, Aubrie O'Rourke 7, Karen E Nelson 7, Sebastian Hiller 4, Nicholas Noinaj 5, Till F Schäberle 3, Anthony Donofrio 1, Kim Lewis 8

Erratum In [Nature. 2020 Apr;580(7802):E3](http://www.ncbi.nlm.nih.gov/pubmed/32269338)

### **Article Abstract**

The current need for novel antibiotics is especially acute for drug-resistant Gram-negative pathogens1,2. These microorganisms have a highly restrictive permeability barrier, which limits the penetration of most compounds3,4. As a result, the last class of antibiotics that acted against Gram-negative bacteria was developed in the 1960s2. We reason that useful compounds can be found in bacteria that share similar requirements for antibiotics with humans, and focus on Photorhabdus symbionts of entomopathogenic nematode microbiomes. Here we report a new antibiotic that we name darobactin, which was obtained using a screen of Photorhabdus isolates. Darobactin is coded by a silent operon with little production under laboratory conditions, and is ribosomally synthesized. Darobactin has an unusual structure with two fused rings that form post-translationally. The compound is active against important Gram-negative pathogens both in vitro and in animal models of infection. Mutants that are resistant to darobactin map to BamA, an essential chaperone and translocator that folds outer membrane proteins. Our study suggests that bacterial symbionts of animals contain antibiotics that are particularly suitable for development into therapeutics.

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MRSA and MRSP in Small Animals

**Western Veterinary Conference 2012**

Patricia Dowling, DVM, MSc, DACVIM, DACVCP

Western College of Veterinary Medicine, Saskatoon, SK, Canada

**Overview of the Issue**

Dogs and cats may be contaminated, colonized, or infected with methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), with implications for the pet's health and as a potential reservoir for human infection. While dubbed "methicillin-resistant" staphylococci, diagnostic labs actually test for resistance against oxacillin or cefoxitin because they are more stable. However, according to the Clinical Laboratory Standards Institute Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee the cefoxitin test is not appropriate for detecting methicillin resistance in *S. pseudintermedius* isolated from dogs cefoxitin breakpoints are not predictive of mecA- mediated resistance. Isolates of *S aureus* or *S. pseudintermedius* should be considered to be methicillin resistant when multidrug resistance is identified, even if susceptibility to cefoxitin is reported.

**MRSA**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a critically important human pathogen that is also an emerging concern in veterinary medicine and animal agriculture. MRSA have been documented in dogs, cats, rabbits, horses, cattle, pigs, poultry, and exotic species, both as a cause of infection and in healthy carriers. The role of animals in the transmission of MRSA in the community is not well defined. However, people who work on farms, own pets, and work in veterinary hospitals may be at greater risk for MRSA colonization or infection because of transmission of MRSA between humans and animals. Changes in the epidemiology of MRSA in one species may result in changes in other species. Exposure of dogs and cats to MRSA was inevitable as the prevalence of community acquired-MRSA increased in people. There are now numerous reports of the concurrent isolation of identical MRSA strains from people and their dogs and cats, with the index case sometimes being the person and sometimes being the pet.

Whether methicillin-resistant or not, *Staphylococcus aureus* is a promiscuous colonizer and pathogen, as it lacks host specificity and can colonize numerous anatomical sites. In a survey of dogs presented for vaccination to the Western College of Veterinary Medicine, 10.2% were positive for *S. aureus*, and of those carrier dogs, 5.9%, 17.6% and 17.6% were exclusively pharyngeally, rectally and nasally colonized respectively. MRSA strains can also contain genes that encode the Panton Valentine Leukocidin toxin (PVL). The PVL toxin has been shown to be responsible for many of the severe clinical symptoms of infection with MRSA, such as furunculosis, severe necrotizing pneumonia, and necrotic lesions of the skin and soft tissues.

It is clear that MRSA exposure is an occupational hazard of veterinary medicine. Most studies of veterinary personnel have indicated relatively high colonization rates compared to the general population. In a study of people attending a veterinary surgery conference in the United States, 17% of small animal veterinary personnel were colonized with MRSA. While factors associated with MRSA colonization in veterinarians have not been thoroughly investigated, a study of equine veterinarians revealed that veterinarians who regularly washed their hands between farms and after handling potentially infectious cases had significantly lower colonization rates, emphasizing the important role of hand hygiene in preventing transmission.

Significant risk factors that have been determined for MRSA infection in small animals are repetitive courses of antimicrobial therapy (especially beta-lactams and fluoroquinolones, duration of stay in a veterinary clinics and having received intravenous catheters or surgical implants. In addition, rates of contact with humans who had been ill and admitted to hospital are higher in MRSA infected pets. Dogs that participate in pet therapy programs in human hospitals and nursing homes raise unique concerns regarding the transmission of MRSA and MRSP. Dogs that are allowed to lick patients or receive treats are at risk of acquiring MRSA through oral exposure to MRSA on patients' skin. Pet therapy animals are also at risk for transient contamination by MRSA in the absence of colonization or infection.

**MRSP**

*S. pseudintermedius* is a normal inhabitant of the skin and mucosa of dogs and cats and can be isolated from the nares, mouth, pharynx, forehead, groin and perianal areas. It can be opportunistic pathogen, causing pyoderma and otitis, urinary tract infections and post-operative wound infections in dogs and cats. Although it was historically reported as *Staphylococcus intermedius* (and still is frequently mis-reported that way) *Staphylococcus pseudintermedius* and not *Staphylococcus intermedius* is the species of the *S. intermedius* group (SIG) which colonizes and causes infections in dogs and cats.

Methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged as a significant pathogen in companion animals. In the past, *S. pseudintermedius* isolates were generally susceptible to penicillinase-stable beta-lactam antibiotics, but since 2006, MRSP has emerged as a significant problem in veterinary medicine. As with MRSA, the methicillin resistance of *S. pseudintermedius* is mediated by the mecA gene that codes for a modified penicillin binding protein (PBP). Normally, beta-lactam antibiotics bind to PBP of *S. pseudintermedius* to prevent cell wall formation. The modified PBP of MRSP has a low affinity for beta-lactams and therefore cell wall formation is not prevented therapy with these antimicrobials. The mecA gene is located on the bacterial chromosome on a mobile element called the "staphylococcal chromosomal cassette" (SCCmec), which can be transferred between different staphylococcal species. Besides mecA, MRSP can also contain a wide range of different antimicrobial resistance genes, making them not only resistant to beta-lactam antibiotics, but also to other classes of antimicrobial drugs, including the fluoroquinolones. Two major clonal MRSP lineages have disseminated in Europe and North America. Isolates originating from North America are often susceptible to chloramphenicol, whereas isolates from Europe are often resistant to chloramphenicol. There are reports of MRSP isolates resistant to all approved veterinary antimicrobials, resulting in pressure to use "last resort" antimicrobials approved for human use.

Many infections with MRSP are (surgical) wound infections, but pyoderma, otitis externa and urinary tract infections are also associated with MRSP. MRSP colonization and infection has been described in dogs, cats, horses, birds and humans. MRSP is cultured more frequently from dogs than cats, with prevalence rates from < 1% up to 30% (dogs in a Japanese veterinary clinic). Transmission of MRSP between humans and animals has been reported. People living in a household with a pet with history of MRSP infection and people working in a veterinary clinic where MRSP-infected pets were treated were infrequently found MRSP-positive. But contact animals and environmental samples are frequently MRSP positive indicating that the household and clinic environments were contaminated and thus the exposure considerable. MRSP can be found in sites where there is little or no physical contact with an infected animal or contact pets (such as the floor under furniture) indicating that hair and epithelial cells carry MRSP to those sites.

**Treating MRSA and MRSP Infections**

Control options for colonized animals have not been well research. The effectiveness of routine application of measures such as disinfecting shampoos to decolonize has not been established and expected effectiveness is particularly dubious for dogs and cats with mucosal colonization of MRSA or MRSP. It is suspected that animals colonized with MRSA or MRSP are at greater risk of developing infections in case of surgical or nonsurgical wounds and when exposed to antimicrobials. But there is no evidence of the effectiveness of antimicrobials to decolonize animals and the use of antimicrobials for this purpose is likely to increase the risk for selection of additional resistance mechanisms. In some countries, veterinary use of "last-resort" antimicrobials, including mupirocin, vancomycin, linezolid and the combination quinupristin / dalfopristin is limited to exceptional conditions or prohibited by law.

In treating MRSA and MRSP infections in dogs and cats, clindamycin may be selected because of its antimicrobial activity and good tissue distribution. .However, an inducible form of clindamycin-resistance may be present in some MRSA and MRSP. These staphylococcal strains appear susceptible on routine antimicrobial susceptibility testing, but resistance is induced during clindamycin treatment, resulting in treatment failure. On a Kirby Bauer plate, strains with inducible resistance to clindamycin are difficult to detect as they appear erythromycin-resistant and clindamycin sensitive *in vitro*. However, if the clindamycin disk is placed next to the erythromycin disk, inducible resistance is detected by a "D" shaped zone of inhibition around the clindamycin disk. Although inducible clindamycin resistance is more frequent in MRSA than in MRSP, it is recommended that the D-test be performed for all erythromycin-resistant isolates that initially test susceptible to clindamycin. In a survey in Saskatoon, inducible clindamycin resistance was found among 78% and 4% of canine and human MRSA and 17% and 25% of canine colonizing and human methicillin susceptible *S. aureus* (MSSA), respectively.

**Summary**

In veterinary medicine, we need:

 Better diagnostic tools for the identification of *S. pseudintermedius*, and to avoid misidentification with *S. aureus* and *S. intermedius*.

 Studies to document whether the long-term colonization of MRSA and MRSP exists and determine efficient ways to decolonize animals that do not rely on antimicrobials.

 More information on the efficacy of various therapeutic strategies in animals infected with MRSA and MRSP.

 Research on non-antimicrobial strategies to treat (surgical) wounds, skin diseases like pyoderma, and otitis externa, the most common conditions associated with MRSA and MRSP.

 Although most infections can be controlled without antimicrobials, there are severe cases that might be life threatening for which no effective veterinary-approved antimicrobials are available for treatment. We need more information on the effects of using human antimicrobials in veterinary patients from the standpoint of resistance.

 Appropriate infection control in pet-owning homes and veterinary clinics to minimize the spread of MRSA and MRSP.

 Specific prudent use guidelines for the appropriate use of antimicrobials in companion animal medicine.

 Surveillance of infection rates of MRSA and MRSP and data on consumption of antimicrobial agents in small animals.

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**Speaker Information**

(click the speaker's name to view other papers and abstracts submitted by this speaker)

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MRSA/MRSP

**British Small Animal Veterinary Congress 2013**

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**Introduction**

*Staphylococcus aureus* is a commonly encountered Gram-positive coccus and considered to be a commensal and probably transient inhabitant of the skin and respiratory tract. It is known to infect a number of species, including humans, dogs, cats and horses. A variety of antimicrobials are effective against *S. aureus*; however, the organism exhibits the ability to rapidly adapt to antimicrobial use, with resistance generally occurring within a few years of introduction of new antimicrobial therapies used for treatment.

Meticillin-resistant *S. aureus* (MRSA) is characterised by the presence of a specific gene (the mecA gene) that produces a protein that alters binding of penicillin and related compounds (i.e., oxacillin, cephalosporins). Several strains of *S. aureus* are recognised, with the USA3000 strain implicated primarily in community-associated MRSA (CA-MRSA) infections. The majority of MRSA infections manifest as skin lesions (abscesses, cellulitis). Necrotising fasciitis and pneumonia infections can also occur. Hospital-acquired MRSA (HA-MRSA) often manifests as septicaemia. While genetic differences are present in the various strains of MRSA, modes of transmission are similar.

MRSP is easily confused with the more common pathogen MRSA (meticillin-resistant *Staphylococcus aureus*). MRSP stands for meticillin-resistant *Staphylococcus pseudintermedius*. Like MRSA, MRSP is resistant to all beta-lactam antibiotics. Despite recent increased interest in MRSP within veterinary medicine, it has been prevalent for longer than may be realised. *Staphylococcus intermedius* (*S. intermedius*) was initially conveyed in 1976, but its taxonomy has been confused since 2005 when the species *S. pseudintermedius* was described. After further research, *S. intermedius* was redefined to *S. pseudintermedius,* the species that colonises and infects dogs and cats. *S. intermedius* does exist as a pathogen in its own right but is extremely closely related and furthermore extremely rare.

**Development of Bacterial Resistance**

While the process whereby bacteria become resistant to specific antimicrobials can be multifaceted, the primary mechanism is through antibiotic pressure, which provides an evolutionary advantage to microbes with specific mutations. Such mutations are often random. However, the overuse or improper use of antimicrobials that results in the survival of primarily the mutated organisms results in survival of the mutated population. Subsequent generations carry on the trait and can also pass the genetic information to other non-mutated organisms. Because many of the antimicrobials used to treat patients have similar modes of activity, organisms can develop that are resistant to many different antimicrobials (multidrug-resistant 'superbugs').

Some resistant strains of *S. aureus* bacteria have been isolated that also show increased resistance to vancomycin. These organisms are referred to as vancomycin-intermediate and vancomycin-resistant *S. aureus* (VISA and VRSA) and have been isolated in human patients with previous MRSA infections and exposure to vancomycin.

**Colonisation and Infection**

MRSA and MRSP can be present without causing clinical symptoms. Such colonisations are common and have been reported in humans and domestic animals. Colonised animals and humans may serve as reservoirs for clinical infections. Pet cats and horses have also been implicated in human MRSA and MRSP infections. In MRSA infections, it has been presumed that the pets were likely to have been infected by their owners and then served as reservoirs for human reinfection once their owners were treated. MRSP, because it is adapted to living on pets and particularly dogs, and can transfer readily to other animals and people, is suited to rapid spread. MRSA infections in humans have also been documented to be associated with MRSA colonisation in their pet dogs. In animals with clinical infections, postoperative and wound infections are most commonly reported. Infections at intravenous catheter sites, urinary tract infections, pneumonia, and skin infections also occur.

**Diagnosis**

All coagulase-positive staphylococci should be identified to the species level using standard bacterial culture and sensitivity testing. Differentiation of *S. aureus* from *S. intermedius*, *S. pseudintermedius*, and *S. scheiferi coagulans* is vital to proper diagnosis and treatment. Meticillin-resistant *Staphylococcus* is then further evaluated based on its susceptibility to oxacillin. While the usefulness of treatment of MRSA- or MRSP-colonised patients has not been well established, the veterinarian will probably consider treating these patients, especially if the owner(s) are at risk for development of MRSA/MRSP infection. Clinic personnel that come into contact with known MRSA- or MRSP-infected or colonised patients should consider evaluation by their own GP.

**Infection-Control Practices**

All clinic personnel should be vigilant in application of proper infection-control practices. Patients that are known or suspected to be infected or colonised with MRSA/MRSP should be treated as infectious and proper precautions taken. Known or suspected MRSA-/MRSP-infected animals should be housed in an isolation area and direct or indirect contact with other animals prohibited. Supplies such as digital thermometers without thermometer covers and bandage scissors should not be used on multiple animals without proper disinfection of the items between patients.

**Hand Protection/Washing**

Gloves and/or disposable protective outerwear should be worn when handling known or suspected MRSA-/MRSP-infected patients. A significant mode of transmission of bacteria is via human hands. Careful handwashing after handling a patient is vital to controlling the spread of infectious disease. The use of automatic water taps and paper towel dispensers is also recommended. Alcohol-based hand disinfectants should be available in numerous locations throughout the veterinary facility and used after each handling of a patient, and it should be ensured these hand disinfectants are effective against MRSA/MRSP.

**Covering Wounds**

Veterinary personnel with open wounds should keep such wounds covered with a bandage until healed. Infection of humans by direct contact is facilitated when the skin barrier is compromised. Personnel with skin wounds of any type may also serve as a potential source of infection for patients.

**Cleaning and Disinfection**

Proper use of routine disinfectants will kill MRSA and MRSP. When transmission of MRSA/MRSP is suspected, it may be useful to vary use of different disinfectants. All cleaning protocols should be evaluated to ensure that acceptable levels of disinfection are obtained while still preserving human health and avoiding potential damage to surfaces. Disinfection of surfaces of all medical equipment is also required and represents an often-overlooked area of housekeeping. Cold sterilisation solutions are also commonly contaminated with bacteria and may serve as a source of infection if improperly used. Ensure that all equipment being cold-sterilised remains in the cold sterilisation solution for an adequate amount of time and those solutions are properly diluted and frequently changed.

**Surveillance**

While routine surveillance is rarely needed in the small veterinary clinic, larger facilities may benefit from development of a surveillance programme. Such programmes can include evaluation of bacterial cultures from the environment, screening of patients and/or staff members during suspected outbreaks, and analysis of data on surgical site infections. Such surveillance programmes may be considered detrimental, as they may reduce hand hygiene/cleaning procedures in non-colonised staff, so whether staff should be screened is debatable.

**Summary**

MRSA, MRSP, and other multidrug-resistant organisms will probably continue to emerge as important zoonotic agents in veterinary practice. In addition to *S. aureus* and *S. pseudintermedius*, resistant strains of *S. intermedius*, *S. schleiferi coagulans*, coagulase-negative staphylococci species, enterococci species, *Acinetobacter*, *Enterobacter*, *Escherichia coli*, and *Pseudomonas* have been identified. All veterinary personnel must be aware of the modes of transmission and methods of control to minimise the negative impact of these infectious agents on the health of patients, owners, and personnel.

**Speaker Information**

(click the speaker's name to view other papers and abstracts submitted by this speaker)

[**Louise O'Dwyer, MBA, BSc (Hons), VTS (ECC), DAVN (Medical & Surgical), RVN**](https://www.vin.com/members/cms/project/defaultadv1.aspx?pId=11374&authorId=52983)

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#### **Email(s) from Dr. Timsy Bhando**

1.

Hi Eden,

Hope you are doing good!

As Eric mentioned, I am game and super excited to help with you with your project questions. All the best for your project and congratulations for taking such a wonderful and important topic.

Wanted to know if it would be okay if I replied to your questions by early next week or if you have an early deadline for the same. Please let me know.

Thanks so much!

Best,

Timsy

#### **!!Expert email reply notes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Name of Expert | Notes from email | Info from email | Date email sent | Date reply received |
| Dr. Till Schaeberle (1&2) | -- | * Antibiotic resistance can be seen as one of the biggest threats to human health * Some people say antibiotic resistance can be seen as a ‘slow pandemic situation’ * Many years, maybe 10 yrs until Darobactin developed into a medicinal drug * No red flags stopping development, but very long process * If Darobactin is effective in humans so far there is no harms on using it for dogs in the future * (more on second email on research document) | Jan 15th, 2021 | Jan 16th, 2021 |
| Dr. Eric Brown | * Await reply of questions from referred expert | -- | Jan 15th, 2021 | Jan 16th, 2021 |
| Dr. Kevin MacAulay | * Reply back with more general questions about articles/answers? | **on research document** | Jan 15th, 2021 | Jan 17th, 2021 |
| Dr. Timsy Bhando (1) | * Await reply of questions | -- | Jan 16th, 2021 | Jan 17th, 2021 |
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