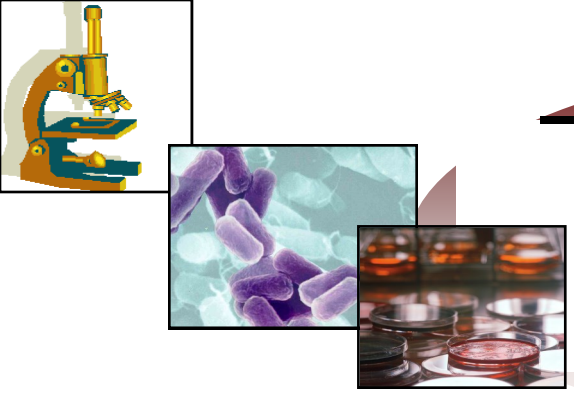

# IBASM NEWSLETTER

**Volume 21, Issue 2**
January, 2019

## *Greetings from the President: Tanya Soule*

I am excited to see all of you at our spring meeting on April 5-6, 2019 at the Brown County Inn in Nashville, IN! Our ASM Branch Lecturer, Dr. Ilhem Messaoudi-Powers from the University of California, Irvine, will be speaking about how alcohol consumption alters our immune defense mechanisms. This year we will also be hosting Dr. Jennifer Brown from the Indiana State Department of Health, who will inform us about several types of zoonotic infections around the state.

The IBASM meeting is a wonderful opportunity for both graduate and undergraduate students to present their work. As a graduate student I attended branch meetings annually and was excited to see that Indiana offered the same opportunities. I encourage you to all submit abstracts for either a poster or oral presentation. For those of you working on a thesis, dissertation, senior, or honor's project this meeting is a great opportunity to present your research to microbiologists for valuable feedback.

While you are at the meeting take a moment to speak with our student representative, Ahmed Hassan from Purdue University. He is eager to represent the students and would love to hear of any ideas you would like to share for the group or future meetings. You may also consider nominating yourself or a friend to be a student representative for the following academic year. See page 2 in the newsletter for more information about this position.

I would also like to remind you that abstracts are due **March 8** while the meeting registration is due **March 1**. Details are in this newsletter for registering, submitting abstracts, and travel.

I would like to thank Dr. Doug Stemke, President-Elect, for organizing the meeting and Dr. Christian Chauret, IBASM Secretary, for always being so helpful with the planning. I look forward to an educational, fun, and successful meeting!

**In this issue:**

## Message from the President-Elect - Doug Stemke

Once again, our colleagues and friends will gather from across the Hoosier state, this time at Brown County Inn (Nashville, IN), to share their discoveries and common interests with fellow microbiologists.

As with previous meetings, many of the findings presented at our regional meeting are the result of access to highly specialized molecular tools and protocols that we use to unlock biological mysteries or solve medical conditions long though unsolvable. For the vast majority of these studies such tools are used responsibly following protocols that follow safe and ethical practices. However, the misuse of such tools, either intentionally or accidently, has the potential to raise serious consequences. Such a misstep became evident last month when it was disclosed at the Second International Summit on Human Genome Editing that Dr. He Jiankui of Southern University of Science and Technology in Shenzhen, China used CRISPR to remove or edit a human gene (note: at the time of this writing this result has not been confirmed). The intent was to remove the receptor gene CCR5 that is essential for HIV's entry into cells expressing this gene. While we can speculate on the researcher's motives, one assumes the primary reason for pursuing this line of research was a genuine desire to provide lifelong protection of the virus in these children. However, the lack of oversight, numerous ethical issues raised including genetic manipulation of a human genome using CRISPR and the potential harm to the offspring themselves emphasize why researchers must follow established scientific and ethical protocols.

We should see this event as an opportunity to pursue conversations in our own fields of microbial research. It was a generation ago when a well-intentioned use and release of a GM Microbe to combat Dutch Elms Disease by Dr. Gary Strobel raised similar concerns of research that proceeded without full scientific and ethical oversight. That noted, it is important to realize that there are well-established protocols to advance novel genetic research that protect the interests of the research and public collectively. Specifically, protocols were developed and published during the Asilomar Conference on Recombinant DNA that developed and set forth guidelines defining how to properly handle GMOs and even when such research should not proceed forward. Since that conference back in 1975 numerous other government agencies, professional meetings, conferences, and institutional ethics committees have sought to refine rules to maintain the balance between the benefits and the potential hazards of similar GM technologies. However, it is up to each of us as users of such technologies to be mindful and knowledgeable of when and how to safely and ethically use those tools that we have inherited.

## Call for Nominations for the IBASM Student Representative

The IBASM Executive Committee is soliciting nominations for a student representative to serve one year (renewable for a second year) on the Executive Committee. Duties include working with the other student representative to serve as a liaison between student members and Executive Committee, submitting a statement to the newsletter twice a year, attending and assisting with the annual IBASM meeting each spring, and bringing ideas for student engagement to the branch. Graduate and undergraduate IBASM student members are eligible. The nominee should be an active student and IBASM branch member through May 2020. Self-nominations or nominations from students, faculty, or staff will be accepted. Please send a 1-2 paragraph statement of interest/nomination, including the nominee's email address, academic year for 2018/2019, and institution, to Tanya Soule at soulet@pfw.edu by May 15, 2019.

# ASM Announces the Peggy Cotter Travel Award Program for Early Career Branch Members

## CALL FOR NOMINATIONS

The ASM Board of Directors has enthusiastically established a new travel award that will enable each ASM Branch to help three early career investigators offset their costs to attend ASM Microbe 2019, June 20-24, in San Francisco. The award is named for our immediate past president, Dr. Peggy Cotter, who is deeply committed to mentoring early stage investigators and providing them with opportunities to launch their careers in science. ASM is committed to partnering with our Branches to provide opportunities for more members to be exposed to all that ASM Microbe has to offer. These awards help facilitate that commitment and honor Dr. Cotter's legacy of helping early career scientists. Priority will be given to new faculty (contract faculty as well as tenure-track) in their first five years of service.

More details, including a definition for early career, information on administration of the award, and selection criteria is explained in detail below. Selection of the recipients will be made by the IBASM Awards Committee. Please send nominations (self-nominations are allowed) to the attention of Professor Dominique Galli, at dgalli@iu.edu by Feb. 10, 2019. Nominations will consist of a current cv, and a one page letter overviewing your recent research and teaching activities that make you a strong candidate to present at ASM Microbe and the IBASM meetings. In addition, a one page support letter should be included from a department chair, school director, or Dean, that acknowledges the research and/or pedagogical activities of the applicant.

**Purpose:** The ASM Peggy Cotter Travel Award Program for Early Career Branch Members provides funds for outstanding early career* Branch members to attend ASM Microbe in 2019.

**Definition of Early Career:** For the purposes of this award, "early career" includes a. Postdoctoral fellows (up to 10-years beyond terminal degree) b. CLS-MLT-MLS Bench Techs (up to 10-years beyond terminal degree) c. Early Career Faculty (up to 10-years beyond terminal degree) d. Early Stage Investigators (ESI's) (up to 10-years beyond terminal degree) e. Other – as determined by Branch, but only individuals who are up to 10-years beyond terminal degree

**Amount and Number of Awards:** Up to 3 awards per Branch; each award is $1650.

**Use of Awards:** Awardees will use the awards to pay for registration, accommodation and travel costs associated with attending ASM Microbe in San Francisco, CA; June 20-24, 2019.

**Abstract Submission to ASM Microbe:** Abstract submission to ASM Microbe is completely independent of the ASM Peggy Cotter Travel Award Program for Early Career Branch Members. Awardees will NOT be guaranteed a poster or oral presentation at ASM Microbe. Awardees of (or applicants to) the Branch awards program may submit abstracts to ASM Microbe (and are encouraged to do so) by the deadline of January 15, 2019, but there is no guarantee that their abstracts will be accepted. Awardees will also be asked to submit a poster abstract (and/or an oral presentation abstract) for the IBASM Branch meeting either in 2019 or for the 2020 meeting.

**Administration of Awards:** Each Branch may identify up to 3 awardees, based on criteria established by the award program and the individual Branch (see below for program criteria). The choice of awardees is solely the responsibility of the Branch. Each Branch will provide ASM HQ with names and contact information of awardees by March 1, 2019. ASM HQ will work directly with awardees to handle processing and distribution of funds. Awardees will be responsible for paying any local, state and federal taxes associated with receipt of cash awards. Federal and State government employees must check with their individual agencies to confirm whether they are permitted to accept cash awards prior to acceptance of any award. Any tax consequences will be the responsibility of the award recipient.

**Travel Arrangements:** Awardees will be responsible for registering for ASM Microbe and making all their own travel arrangements to attend the meeting. The award funds will be distributed at or after ASM Microbe.

## Message from the Student Representative

Dear IBASM student members,

I am Ahmed Hassan, the student representative of the IBASM. I hope you had a great break and ready to start the new semester fully charged. This year our IBASM meeting will be on April 5th and 6th in Brown county, Nashville, IN. Please make sure to participate and showcase your research either through an oral or a poster presentation. The meeting is a great opportunity, especially for students, to meet and network with other colleagues in the field of microbiology. In addition, we have a vacant spot for a student representative so please consider applying. It has been a great experience for me and I am sure you will enjoy and benefit from it. I will be happy to hear your suggestions and communicate them to IBASM board members. My email is Hassan23@purdue.edu, please feel free to contact me anytime.

I wish to see you all in the meeting.

Take care,
Ahmed

# IBASM 2019 Call for Abstracts
# Student Poster Competition

The abstract submission form is included here but will be distributed by email separately as a word document. We will be utilizing 4x4 sq.ft. tri-fold styrofoam poster boards and each student is limited to one board. Push pins will be supplied but it wouldn't hurt to bring some extras in case we run short. You may participate in both oral (limited # of slots available) and poster sessions but you will only be judged for an award in the poster session. Awards will be presented in the following divisions: Undergraduate, MS graduate and Ph.D. graduate. Post-Doctoral Fellows or post baccalaureate students are welcome to participate in either session but are not eligible for the award competition.

**Students will be judged in 5 categories:**

Professional Appearance: Jeans and sweat pants are unacceptable; torn, dirty, or frayed clothing is unacceptable. Business casual dress is the standard dress code. (20 points)

Scientific Thought: Is there a clear hypothesis? Are the goals of the study defined? Were data correctly analyzed? Were statistical analyses performed? Did a logical conclusion result? (20 points)

Creativity: Was the topic original? Is there anything new in the approach to answering the question? Were new methods developed? (20 points)

Thoroughness: Was the study as complete as possible? Does the student understand the background material? Were subject headings (e.g. Introduction, Materials & Methods, etc.) presented? Is the student aware of the drawbacks of the study? (20 points)

Presentation (poster): Were the results/conclusions clearly presented? Was the student's verbal expression clear and concise? Was the student able to answer questions? How well did the poster convey the information? (20 points)

**ABSTRACT FORM FOR THE 2019 IBASM ANNUAL MEETING**

Complete all appropriate boxes of this form (downloadable version http://ibasm.iweb.bsu.edu/) and email it on or by **March 8th 5:00 pm** to Dr. John McKillip at jlmckillip@bsu.edu. **Late submissions will not be accepted**. Abstracts should be prepared according to the National ASM guidelines which are included below; *an example is also provided below for format.* All abstracts should include the title, author(s), and institutional address. Accepted abstracts will be published in the meeting program if submitted by the deadline. Limited funding will be available to subsidize lodging and food for student presenters when requested in the registration form.

Presenting author information:

| | |
|---|---|
| Name: | Phone: |
| Address: | Fax: |
| Subject Category: | Email: |

Are you a student presenter? ☐ Yes or ☐ No (check one)

☐ Oral and/or ☐ Poster presentation (check applicable boxes)

If you are not selected for an oral presentation, are you willing to present a poster?

☐ Yes ☐ No ☐ Does not apply

Check if presenting author is a student competing for: ☐ Undergraduate ☐ MS Graduate or ☐ Ph.D. Graduate Student Award (a short paper is required from award winners). If competing for an award the student must present a poster. (If left blank, the student will not be judged in the competition).

Check if presenting student will also be presenting at the 2018 ASM General Meeting: ☐

Are you competing for the national travel award to the 2018 ASM General Meeting? ☐ Yes ☐ No

**Title:**

**Authors:**

**Affiliation(s):**

**Keywords:**

**ABSTRACT**

**Example Abstract (for reference in formatting yours):**

# Characterization of Bacterial Isolates from Chronic Conjunctivitis in Dogs and their Ability to form Biofilm *in Vitro*

A. Hassan*[1], W. Younis[1], G. K. Hammac[1], J. Stiles[2] and M. N. Seleem[1].

[1]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907, USA

[2]Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN 47907, USA

Biofilms are responsible for 80% of microbial infections which develop in the body and bacterial biofilms on tissue surfaces and chronic wound constitute an ever-increasing threat to human and animal health and place a significant burden on healthcare systems. Chronic bacterial infections in veterinary medicine are commonly reported but association with biofilms is rarely considered. Hence, the objective of this *in vitro* study was to investigate the association between biofilm formation and clinical cases of chronic conjunctivitis in dogs. Twenty bacterial isolates identified at the Indiana Animal Disease Diagnostic laboratory from specimens collected from chronic conjunctivitis in dogs admitted to the small animal teaching hospital at Purdue University were included in the study. Standard methods including examination of colony morphology, hemolysis, biochemical tests, and fermentation of maltose, trehalose and lactose, were used in addition to matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry to identify isolates. In addition, biofilm formation was evaluated using two different methods: Congo Red Agar (CRA) and a microtiter plate assay followed by crystal violet staining. The microtiter plate assay was more sensitive in detecting biofilm forming isolates than was CRA. We were able to detect biofilm formation in 100% of the isolates using the microtiter plate assay, while using CRA we were able to detect biofilm in only 50% of the isolates. In this study, the clinical isolates were confirmed to have biofilm-forming capability, thereby further complicating the therapeutic conditions and outcomes. Furthermore, the microtiter plate assay was shown to be more sensitive than CRA for detecting biofilms and should be considered as a standard technique for detection.

Key words: biofilm; canine; conjunctivitis; MALDI-TOF

# From the Desk of John McKillip...Educational Representative

## ABSTRACT GUIDELINES BASED ON AMERICAN SOCIETY FOR MICROBIOLOGY (ASM) REQUIREMENTS

### SUMMARY OF SUBMISSION STEPS

**Step One: Submit the 2019 IBASM Membership Application/Renewal and Registration Forms.**

**Step Two: Affirmations**

- Names of all the authors (primary and co-authors) will appear on the abstract.
- The submitted abstract representing your research has not been accepted for publication in a journal or in an international scientific journal based on the date submitted.
- Upon acceptance of the abstract, the prepared poster (see guidelines) will be placed on the scheduled day and time for viewing and only be removed after the scheduled time.
- Any changes in contact information should be corresponded with IBASM.

**Step Three: Title**—Please use a short and concise title that indicates the content of the abstract.

- Capitalize the first letter of each word except prepositions, articles and species names.
- Title is not included in the total character count of 2000.
- Do not place a period at the end of your title.
- Do not place hard returns in your title.
- Italicize scientific names (example: "Staphylococcus aureus" will appear as *Staphylococcus aureus*). For therapeutic agents, only generic names may be used (NO trade names are permitted in abstract titles).
- A title of 10 words will be appropriate.

**Step Four: *Primary Presenting Author*, Co-Authors, Affiliation**

- Authors, groups and institutions and spaces are not included in the 2000-character limit.
- Author's names will be displayed using first initial(s) and full last name. Presenting author will be printed with an asterisk (e.g. J. Smith*, W. S. Brown, and R. A. Jones).
- Each institution and author will be referenced with superscript numbers and include the institution's city, two letter state/province abbreviation and country.
- Please note that IBASM will correspond with the presenting author only. Changes in the presenting author must be communicated to IBASM. It is the responsibility of the presenting author to contact all co-authors with the disposition and scheduling of the abstract. The complete address of the presenting author is required in order to assure that correspondence arrives promptly and easily.

**Step Five:** ***Keywords***

- Keywords are completely independent of each other and should be able to stand alone in the index.
- Words should be lowercase, except for genus names and proper nouns. For Greek characters, please spell out names.
- Organisms will be italicized in final publications.
- Enter up to three keywords.

**Step Six:** **Abstract Text** *(included in the 2000-character limit - spaces are not counted)*

- Your abstract may have up to 2000 characters, which does not include the title, authors, affiliations, and keywords. Spaces are not counted. Do NOT include abstract title, authors or keywords in the abstract text.
- Abstract text may be submitted using either of the following methods: Copy/paste or direct entry keystrokes.
- Your abstract may be written without the use of the following bolded headers **(Background, Materials, Results, Conclusion)**. However, your abstract should include a one to two sentence introduction, the description of the methods used, the results obtained, and a conclusion with inclusion of its significance.

# POSTER GUIDELINES FOR THE SPRING 2019 IBASM ANNUAL MEETING

Brown County Inn, Nashville, IN. April 5/6th, 2019

1. ABSTRACT
    - Including an abstract on your poster is recommended and expected by most readers. Ideally, this would be the exact same abstract submitted initially and in the program booklet, although if any new (and vital) data are obtained immediately prior to the meeting, these results may be included on the poster, which could alter the wording of the abstract slightly, and this is acceptable.
2. INTRODUCTION
    - The introduction should give the reader a solid foundation on which to base their understanding of the rest of the poster. It should convey: the importance of the research, the problem you are trying to solve, why the research is necessary, your approach to the problem and the logic behind it, and your **hypothesis**.
    - Do not be verbose; the best Introduction sections are 2-3 paragraphs.
3. METHODS
    - Give enough detail so the reader understands what was done, but do not explain every step. Assume basic knowledge. Be sure to address what and how your statistical analyses were completed and your significance level (s).
    - Present methods in a way that is easy to follow. Cite relevant references.
4. RESULTS
    - Try to make your figures, if possible, in black and white or colors that are colorblind friendly.
    - All figures and graphics should be as high resolution as is possible and practical
    - DO NOT DISCUSS your results in the Results section. You should merely state the observed results.
    - All figures and tables should be self-explanatory; that is, the legend or caption should thoroughly but succinctly explain what the reader is looking at, and should include all relevant labeling, statistical analyses, and scale bars as appropriate 5.
5. DISCUSSION
    - This is where you interpret your results and discuss them.
    - Refer to all of your figures when discussing the corresponding data!
    - State your conclusions and future directions.

6. REFERENCES

- Space is limited so you should only include your most relevant references.
- · Author(s) last name and author(s) initial of first name. Title. Journal Name, Month and Year. Volume #(Issue #): pages. Digital Object Identifier (DOI).
- Law, J. and Gomez, G. 2016. Poster guide for the 2016 IBASM annual meeting. *Journal of Exampleology*, January 2016. 10(1):103-107. doi: 10.1007/s02253-072-1135-4

7. ACKNOWLEDGMENTS

- It is always important to recognize those who helped you analyze data, conduct some of the research, your advisor/mentor who supervised the research, and especially those who provided you with funding, supplies, or organisms.
- Do not acknowledge coauthor(s) of your poster.

8. GENERAL NOTES

- Have poster dimensions set at 40 inches by 24 inches.
- Avoid having font smaller than 24 in Times New Roman (for example).
- Make sure the formatting scheme is consistent!
- The poster should stand alone- it should tell the story of your research without you having to be there.
- Poster sections should be well organized and have a natural flow to them.
- All figures and sections should be properly titled.
- Make sure all edges line up properly.
- Give at least a 1 inch page margin on all sides or you may find your print off eating into your texts and figures.

# *2019 IBASM Spring Meeting*

**April 5-April 6, 2019**

**Brown County Inn, Nashville, IN**

TENTATIVE AGENDA

**Friday April 5, 2019**

| | |
|---|---|
| 5:00-7:00 PM | Registration |
| 7:00-8:00 PM | Dinner (On your own, several local establishments to choose from) |
| **8:00-9:00** PM | **IBASM Branch Lecture**- Dr. Messaoudi-Powers<br>Department of Molecular Biology and Biochemistry, School of Biological Sciences, University of California Irvine<br>"How Alcohol Consumption Alters Our Immune Defense Mechanisms" |
| 9:00-10:00 | Welcome Reception with Poster Viewing |

**Saturday April 6, 2019**

| | |
|---|---|
| 7:30-8:30 | Breakfast and Registration |
| 8:30-10:30 | Poster Viewing and Judging |
| 10:30-11:30 | **Guest Lecture**- Jennifer Brown<br>Indiana State Department of Health<br>"One Health in Action: Investigation of Zoonotic and Vector-Borne Diseases." |
| 11:30- 12:00 | Student Oral Presentations |
| 12:00-12:30 | IBASM Business Meeting |
| 12:30-1:30 | Lunch |
| 1:35-2:30 | Student Oral Presentations |
| 2:30-3:15 | **IBASM Research Award Recipient Lecture** |
| 3:15-3:45 | Announcement of Student Award Winners<br>Closing Remarks |

**Directions and Parking.**
The Inn is near downtown Nashville, IN at the convergence of the county roads 46 and 135.

# IBASM Annual Meeting Registration and Meal Reservation Form

## Brown County State Inn, Nashville, IN April 5th and 6th, 2019

Please use this form (downloadable version : https://indianaibasm.weebly.com/) to register for the IBASM meeting and to reserve your room. The meeting registration fee is $30 for regular members and $7 for student members. You must be an IBASM member to participate in the meeting. Family members are encouraged to attend and do not have to pay registration fees. Upon completion, email (or mail) this form to Doug Stemke (stemked@uindy.edu) no later than **March 1, 2018**. If necessary, forms may also be mailed to Doug Stemke at the address given on the Membership Form below. Payment can be in the mail with a check payable to IBASM (do not send cash) or provided at the meeting (cash or check). Registrations received after March 5 will be subject to a $7.00 late fee (regular members) or a $4.00 late fee (student members). Please feel free to contact Doug Stemke at the email address provided above if you have any questions. **Remember, meeting abstracts are due March 8, 2019**.

Please fill in the requested information.

| | | |
|---|---|---|
| Name: | #Adults: | #Children: |
| Address: | | |
| Institution:<br><br>Phone: | <br><br>Fax: | <br><br>Email: |

If you are not a member, you will need to become a member and include your dues with your payment for the meeting.
2018 IBASM member: ☐ Yes ☐ No

Please indicate which sessions you plan to attend:

☐ Friday evening session ☐ Saturday morning session ☐ Saturday afternoon session

If you are a student presenter, do you request travel assistance? ☐ Yes ☐ No

**Lodging and Meals:** For the IBASM Brown County Inn Reservations:

The group rate for overnight rooms for April 5-6, 2019 is $109 + tax per night for a room with two queen bed.
For reservations directly call the inn at **(812) 988-2291**. The Inn is near downtown Nashville, IN at the convergence of the county roads 46 and 135.

For information about the inn: www.browncountyinn.com

**Please be prepared to pay individually (cash or card) for each meal at the Inn.**

**Payment:**

Registration: ☐ Member ($30) ☐ Student ($7) $_____

Dues (if applicable; see following page): ☐ Non-student ($15) ☐ Student ($5) $_____

Late fees (if applicable) $_____

Total Enclosed (mail or pay on-site) $_____

## 2019 Membership Application/Renewal

*If you have not done so already, it is time to pay your IBASM dues for 2019. You can do it either online when you pay your dues to the ASM National Organization (www.asm.org) or by using this form (downloadable version https://indianaibasm.weebly.com/). Dues are $15.00 for non-students and $5.00 for students (per year). Please return the completed form with payment to either the IBASM meeting (cash or check) or by mail with a check (do not send cash), payable to IBASM, to:*

Dr. Douglas Stemke
Department of Biology
University of Indianapolis,
1400 E Hanna Ave
Indianapolis, IN 46227
Phone: (317) 788-2169; e-mail: stemked@uindy.edu

Please check: □ New Member Application or □ Renewal for 2019

Please check: □ Student Member in 2019 ($5) or □ Full Member in 2019 ($15)

Please check: ☐ Dues paid to IBASM directly or ☐ Dues paid online at www.asm.org

Name:

Current Position & Title:

Institution:

Mailing Address (new address Yes / No?):

Phone:

Email:

Fax:

National ASM Member # (if applicable):

**Background**

Highest Degree:

Institution:

Professional Interests:

## Second Place Graduate (MS) Division Winner

# Evaluation of Antibiotics on Immature and Mature Endodontic Bacterial Biofilm

**Ji Yoo Lee**[1], Ghaeth H. Yassen[1], Ygal Ehrlich[2], Kenneth J. Spolnik[2], & Richard L. Gregory[3]

[1]Indiana University School of Dentistry, Indianapolis, IN

[2]Department of Endodontics, Indiana University School of Dentistry, Indianapolis, IN

[3]Department of Biomedical and Applied Science, Indiana University School of Dentistry, Indianapolis, IN

**Background/Overview:**

When an untreated cavity progresses to a severe lesion with a necrotic pulp, it is generally treated with conventional root canal therapy, in which disinfecting canals, removing smear layer, and sealing inside of the pulp chambers with gutta percha are performed to save the tooth (1). Recently, regenerative endodontics (RE) has become an innovative way to treat infected root canals of immature permanent teeth (2). It has been suggested that regenerative endodontic therapy replaces impaired pulp tissues and preserves stem cells to retain functionality, thereby enhancing tooth longevity (3). Also, this treatment procedure allows continued root development and hard tissue deposition on the dentinal wall, strengthening the roots. To successfully accomplish outcomes of the treatment procedure, a total disinfection of infected root canals and preservation of mesenchymal stem cells present in either the apical papilla or dental pulp is essential (4). For these purposes, RE uses antibiotic intracanal medicaments including triple and double antibiotic paste (TAP containing minocycline, ciprofloxacin and metronidazole, DAP containing ciprofloxacin and metronidazole; 2,5). In necrotic pulps, several types of bacterial species may exist (6). *Enterococcus faecalis* and *Porphyromonas gingivalis*, which are commonly found in infected root canals, form biofilm to evade the immune response of the host or protect themselves against antibacterial effects provided by medicaments (2,7). Previous studies have demonstrated inhibitory effects of TAP and DAP on biofilm formation of *E. faecalis* and *P. gingivalis* (7). However, investigators have observed that these antibiotic medicaments may have toxic effects on the survival of mesenchymal stem cells present in pulp tissues at high concentrations (8). It is important to investigate the appropriate concentration of the medicament that is safe to use for the preservation of stem cells essential for pulp regeneration. Furthermore, research has proposed the following challenge regarding the use of TAP: it contains minocycline, an antibiotic that can cause tooth discoloration (7). Due to absence of minocycline, the use of DAP may be more beneficial than TAP application in this regard.

**Specific Aims:**

For this study, multiple species of endodontic-related bacteria were collected from pediatric and adult patients with necrotic pulps. We have differentiated the testing samples into two mixed species groups, the first group was collected from a necrotic pulp of a pediatric patient (termed immature) and the second group was collected from a necrotic pulp of an adult patient (termed mature). This particular immature preparation contained significantly more *E. faecalis* than the mature preparation (unpublished data). The use of these two bacterial samples is innovative due to the mixed species in each preparation. No studies have reported the effect of DAP on mixed species from clinical isolates. The aim of the present study was to examine the antibacterial effect of DAP against biofilm formation of immature and mature mixed endodontic species preparations. It was hypothesized that DAP will be effective in reducing biofilm formation of these clinical isolates and that there will be differences in the sensitivity of the isolates due to the different species present in each preparation.

## Methods:

Bacterial strains and media: The bacterial species used consisted of the clinical immature and mature biofilm preparations previously collected from endodontic patients under full IRB approval. The immature and mature species were collected using paper points from pediatric and adult patients, respectively, with necrotic pulps. The species were stored at -80°C in tryptic soy broth (TSB; Acumedia, Baltimore, MA, USA) with 20% glycerol, before use. Brain heart infusion broth supplemented with yeast extract, vitamin K, and hemin (BHIY; Becton, Dickinson and Company, Franklin Lakes, NJ) was used to culture the species. The bacteria were cultured for 24 h at 37°C in an anaerobic environment.

Preparation of medicament: DAP (CHAMPS Medical, San Antonio, TX) was purchased in powder form. Distilled water was added to the powder, and the mixture kept in a water bath and thoroughly mixed prior to use. The original concentration of the mixture was adjusted to 40 mg/mL. BHIY was used to dilute DAP to different concentrations ranging from 0.0024 to 20 mg/mL.

Effect of DAP on initial biofilm formation: In sterile 96-well microtiter plates (Fisher Scientific), 10 µl of overnight cultures of the immature and mature bacterial species and different concentrations of DAP (190 µl; 0-20 mg/mL) was added at the same time. Negative controls were prepared without DAP treatment. The incubation period was 72 h.

Effect of DAP on established biofilm formation: In this set of experiments, biofilm formation was completed prior to adding DAP. The immature and mature species were plated without DAP in the 96-well plates for 48 h to establish biofilm. Then, different concentrations of DAP (0-20 mg/mL) were added to the designated wells and incubated for 24 h.

Crystal violet staining assay: After incubating both DAP-treated bacterial species and negative controls, a well-described crystal violet staining assay as described by earlier publications from this laboratory was used to determine the amount of biofilm formation.

Minimum bactericidal concentration: The minimum bactericidal concentration (MBC) is the lowest concentration of an agent that kills microorganisms (9). To identify the MBC, immature and mature species to be treated with different concentrations of DAP (0-20 mg/mL) and negative controls were streaked on blood agar plates and incubated for 24 h. The presence or absence of colonies allowed determination of the MBC.

Statistical analysis: Each experiment was performed individually at least three times. Data were analyzed using SPSS software (version 16.0; SPSS, Chicago, IL, USA). Statistical significance was determined by two-way ANOVA to compare the means of DAP-treated immature species and DAP-treated mature species. P values of $<0.05$ were considered significant.

## Results:

Initial and established biofilm formation: DAP significantly decreased initial biofilm formation of mature species from 0.0098 to 10 mg/mL (Fig. 1).

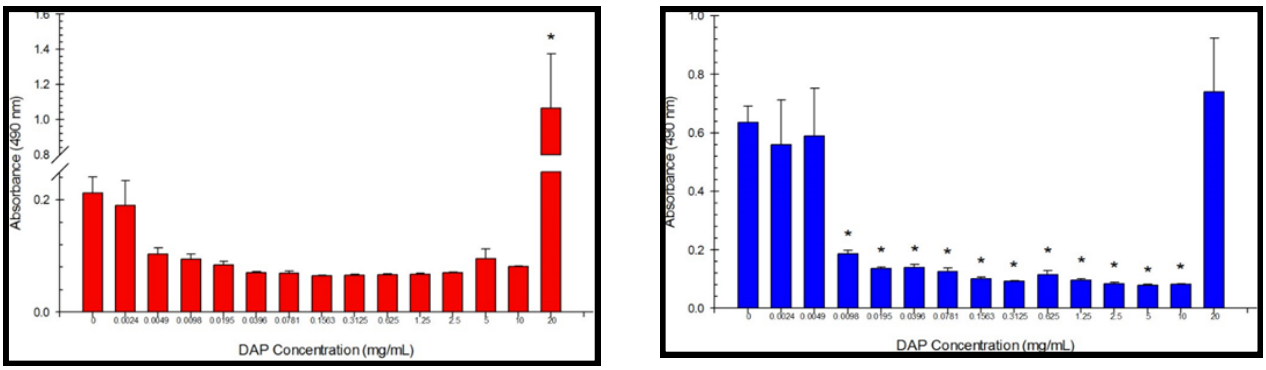


**Fig. 1.** Biofilm formation of immature (red bars) and mature (blue bars) species with DAP added at the same time. Significant differences ($p < 0.05$) compared to the 0 DAP control are indicated by *.

However, no significant inhibition of initial biofilm formation by immature species was observed at any concentration of DAP. DAP significantly attenuated the established biofilm formed by mature species at 0.0396 mg/mL, whereas no significant inhibition of biofilm formation by immature species was observed at any concentration of DAP (Fig. 2).

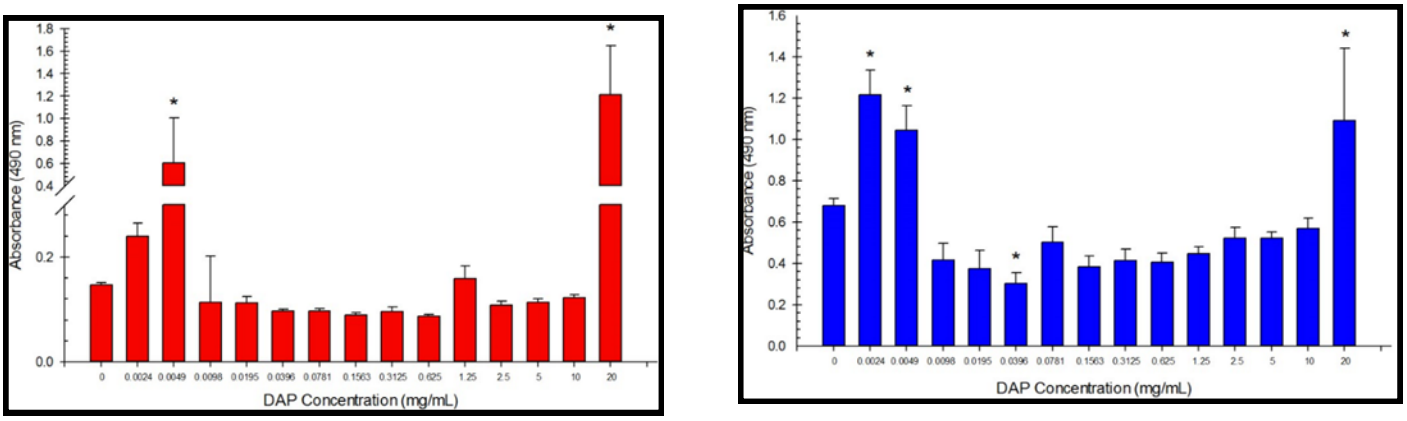


**Fig. 2.** Biofilm formation of immature (red bars) and mature (blue bars) species with DAP added 48 h post-incubation of bacterial species. Significant differences ($p < 0.05$) compared to the 0 DAP control are indicated by *.

## CONCLUSIONS

Our preliminary results provide insight into pulp regeneration, which is associated with root canal infections that can be eliminated using different chemical treatments including DAP. Treatment with DAP significantly attenuated the biofilm formation induced by oral bacterial species obtained from adult (mature) endodontic patients. However, we have also observed an arithmetic reduction of biofilm cells formed by pediatric (immature) patients with necrotic pulps.


## KEY WORDS

Pulp regeneration, double antibiotic paste, biofilm


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# Second Place Undergraduate Division Winner

## Investigation of β-lactamase Expression in *Enterobacter sp*. from Canada Goose (*Branta canadensis*)

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## Introduction

Antimicrobial resistance (AMR) is a growing problem; according to the CDC, 2 million patients at hospitals become infected with resistant bacteria (1). This increase in AMR is due to increased use of antibiotics in clinical and agricultural settings, selecting for resistant bacteria. In some cases, bacteria are resistant to β-lactam antibiotics due to production of β-lactamases. Class C inducible β-lactamases are becoming more frequent in clinical strains. In the clinical setting, this type of β-lactamase is concerning as these strains are being selected based on the use of inhibitors such as clavulanic acid that were previously effective. *Enterobacter sp.* are targeted here due to naturally occurring chromosomal AmpC β-lactamases found in most strains (7).

*Enterobacter sp*. (C21,G06 C123,A06 C97,C102, C13) were isolated from Canada goose (*Branta canadensis*) feces in Lisle, IL and were chosen based on varying AMR phenotypes. Previously, C13 was found to have a novel β-lactamase, previously named ACT-11. By isolating periplasmic extracts from these strains, various tests were performed including nitrocefin activity assay and mass spectrometry analysis in order to identify the possible presence of ACT-11 and other β-lactamases in the environment. In addition, these five strains were subjected to whole genome sequencing to thoroughly explore the genetic diversity of Class C and other β-lactamases found in these environmental isolates.

## Methods

**Disk Diffusion:** Antimicrobial Susceptibility Testing performed per Clinical and Laboratory Standards Institutes guidelines with control strain ATCC 25922 (3,4).

**Periplasmic Extract:** Bacteria were grown in 3mL LB +β-lactam overnight in shaker. 100µl of culture was transferred to 50 mL of LB + β-lactam and shaken for 4 hours. The fresh culture was then centrifuged and washed and resuspended in sodium acetate. A freeze-thaw method was used to obtain periplasmic extracts by alternating between a 37°C water bath and a dry ice/ethanol bath, five times for 10 second intervals. The samples were centrifuged, and the supernatant recovered.

**Nitrocefin Assay:** A 96 wells plate was filled with 30µl nitrocefin. Periplasmic extract was pipetted in and color change was observed over a 5 minute interval as seen in **Figure 1** to check for β-lactamase activity in the extracts.

**MS/MS:** 50µl of periplasmic extract was added to a 100µl urea tube. Bond breaker was then added to samples and they were incubated a 37°C for 30 minutes. The samples were then split in half and iodoacetamide was added then the samples incubated for 20 minutes at 37 C°; either chymotrypsin or trypsin was added to samples in a 1:10 weight ratio (trypsin:protein) and reacted for 12 hours at 37°C. Addition of formic acid stopped the reaction. The samples were filtered using microcentrifuge filters and analyzed by a Orbitrap Elite™ Hybrid Ion Trap-Orbitrap Mass Spectrometer.

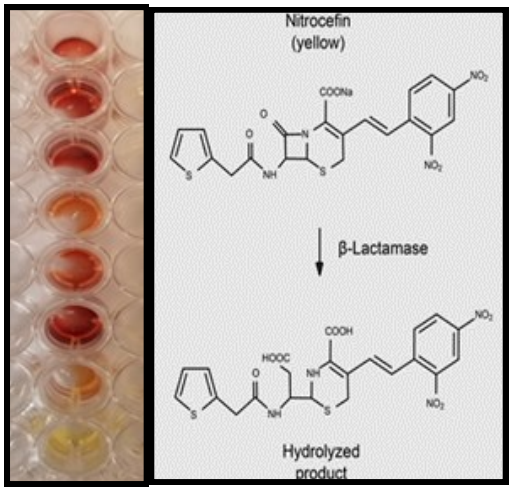


Figure 1: Nitrocefin Hydrolysis

## Results and Conclusions

From the disk diffusions four phenotype groups were identified in **Table 1**. In C102 resistance was inhibited by clavulanic acid. C13 was AmpC like with resistance to cefoxitin and no inhibition with clavulanic acid. G06 C123 and A06 C97 showed extended spectrum cephalosporin resistance. C21 also showed extended spectrum cephalosporin resistance but was not resistant to ceftazidime alone. In the future we will test the other 60 strains in our library and group them based on these phenotypes for further study.

### Table 1: Disc Diffusion Results

| Strain | AM | AMC | FOX | PIP | CTX | CTX/ CLA | CAZ | CAZ/ CLA | TET | CIP |
|---|---|---|---|---|---|---|---|---|---|---|
| IU 429 C21 | 6 | 6 | 6 | 16.5 | 14 | 14 | 21 | 12 | 20 | 30 |
| IU 438 G06 C123 | 6 | 6 | 6 | 17 | 11 | 11 | 11 | 12 | 21 | 30 |
| IU 439 A06 C97 | 6 | 6 | 6 | 14 | 12 | 14 | 11 | 11 | 20 | 37 |
| IU 463 C102 | 7 | 18 | 24 | 21 | 30 | 36 | 32 | 31 | 23 | 33 |
| IU 488 C13 | 6 | 6 | 6 | 27 | 27 | 26 | 30 | 29 | 18 | 36 |
| 1336 (ATCC 25922) | 17 | 20 | 27 | 30 | ND | ND | 33 | 29 | 24 | 40 |

Red Resistant, Yellow Intermediate, Black susceptible, ND not determined; *control not within range

Mass Spectroscopy results are shown in **Table 2**. Of 5 tested strains, 4 resulted in hits above our confidence threshold. Strain C21 was identified to be AmpC β-lactamase from the complete Uniprot database. Peptide sequences were used with NCBI's standard protein blast to identify the proteins. For sample G06 C123, we have two results with similar coverage and confidence. The two results differ only by 3 peptides, therefore we were unable to distinguish between them. For samples G06 C123, C102, and 488 we achieved protein identification by first obtained the gene sequence and then identifying the corresponding protein expression with Mass Spectrometry. Through whole genome sequencing a variety of sequences for putative β-lactamase enzymes were identified including CMY, ACT and partial genes including metallo β-lactamases. It is possible that these strains may code for multiple β-lactamases not all of which are expressed, this could be the basis of discrepancies between our phenotypic results and our targeted mass spectrometry approach.

Future directions include growing the bacteria with cefoxitin to induce AmpC expression, and perform nitrocefin kinetic assays on the periplasmic extracts with and without EDTA to check for potential metallo β-lactamase activity and determine the activity of each periplasmic extract.

**Table 2: Peptide Mass Fingerprinting Results**

| Strain | Accession number | Confidence | Coverage | Description |
|---|---|---|---|---|
| G06 C123 | WP_047954938.1 | 100% | 98.43% | Class C β-lactamase |
| G06 C123 | WP_044858129.1 | 100% | 98.43% | Amp C β-lactamase |
| C102 | WP_044858129.1 | 100% | 99.74% | ACT family cephalosporin-hydrolyzing class C β-lactamase |
| C21 | G8LIHO | 86% | 85.04% | Amp C β-lactamase |
| 488 | WP_020884810.1 | 100% | 81.40% | Metallo-hydrolase β-lactamase |

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# MICROBIOLOGY IN THE NEWS

https://www.eurekalert.org/bysubject/index.php?kw=33

## Where will the world's next Zika, West Nile or Dengue virus come from?

*Nature Communications*

**January 04, 2019**

Scientists from the University of California, Davis, have identified wildlife species that are the most likely to host flaviviruses such as Zika, West Nile, dengue and yellow fever. They created a global flavivirus hotspot map from their findings.

## Montana State research shows gut microbiome protects against acute toxicity

*Nature Communications*

**January 04, 2019**

Research conducted at Montana State University shows that microbes in the human gut play an important role in protecting against arsenic toxicity, a problem that affects an estimated 200 million people who are exposed to arsenic through contaminated drinking water.

A better understanding of how the microbiome protects against toxins like arsenic could benefit communities or villages with contaminated water sources through probiotic or other microbiome therapies, especially since it is not always practical or possible to replace a water source.

## Could this widely used food additive cause celiac disease?

*Frontiers in Pediatrics*

**January 03, 2019**

Celiac disease is none of these things. It is an autoimmune disorder, where gluten triggers the immune system to attack the gut. It is common, lifelong, and can seriously harm health - but nobody knows for sure what causes it. Now a review says a common food additive could both cause and trigger these autoimmune attacks, and calls for warnings on food labels pending further tests. This additive is a bacterial enzyme that is used to improve food texture and shelf-life.

# Important Dates

| | |
|---|---|
| March 1, 2019: | Registration form due for Annual IBASM meeting |
| March 8, 2019: | Abstract form due for Annual IBASM meeting |
| April 5-April 6, 2019: | Annual IBASM meeting, Brown County Inn, Nashville, IN |
| June 20-24, 2019: | ASM Microbe 2019, San Francisco, CA |

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