# Methods around **mechanisms**

**Peter Panizzi,** Assistant Professor at the Harrison School of Pharmacy, Auburn University, outlines his group's efforts to lay the groundwork for the development of non-invasive methods to detect *Staphylococcus aureus* infections

**What is your ultimate aim of your project, 'The Pathobiology of *Staphylococcus aureus* Endocarditis'?**

The ultimate goal of the project is to come up with a better understanding of the underlying mechanisms that *S. aureus* uses to cause disease in humans. Through this new understanding, effective mechanism-based detection methods can be developed to non-invasively detect *S. aureus* infections.

**What are the limitations of current diagnostic methods of endocarditis? How will your study into the pathobiology of *S. aureus* aid in improving those methods?**

Currently, endocarditis is detected by transaesophageal echocardiogram, which requires a hospital stay and several imaging sessions in order to determine whether a patient requires heart valve replacement. This technique does not result in a definitive answer, meaning it is often subject to interpretation of the dataset by a trained radiologist. The diagnostic signs that the physician is looking for are a lack of appropriate motion in the valve, or the presence of bacterial-platelet-fibrin masses in certain areas of a rapidly opening and closing valve. Our results so far indicate that through the use of systemic injections of bacterial-specific probes we can detect endocarditis in mouse models of the disease and monitor treatment efficacy.

**Can you provide a background of your research methodologies and how they have developed?**

I learned most of my skills for protein modification and probe design by conducting projects on other disease states, namely atherosclerosis, wound healing and blood coagulation. Despite this varied background, the technology, as far as imaging methodology is concerned, does not really change and differences stem from the kinetics of the disease development (ie. how long it takes for disease onset). The advantage or disadvantage, depending on your viewpoint, for studying staphylococcal infections like endocarditis is that the disease progresses relatively fast compared to the onset of atherosclerosis or cancer, which is in the order of weeks to months in mouse models.

**How optimistic are you that new adjunctive therapies can be developed?**

Aside from the development of new imaging probes and methods for the detection of *S. aureus* infection *in vivo*, it is also very possible that our studies will allow us to discover elements necessary for the development of endocarditis. Once we have a detailed understanding of the biology behind these processes, we can begin the task of interfering with these elements as adjunctive therapies. These adjunctive therapies would obviously be in addition to the normal antibiotic regimens that are more traditionally used treat bacterial infection and would be complemented by our new imaging agents.

**Where would you like to take this research in the future?**

Our ultimate goal is to be able to develop an agent that can be used in a clinical setting. We have plans to expand studies of the efficacy of these probes in more clinically relevant models, thus moving our model and studies from mouse to minipig. Auburn University is particularly suited for these studies; it has a College of Veterinary Medicine and a recently established Swine Research Centre. Extension of our endocarditis studies into minipigs, and expansion of our probe arsenal, is being done in collaboration with Dr Matthias Nahrendorf of Massachusetts General Hospital and Harvard University, and is supported by a multi-PI R01 (1R01HL114477) grant from the National Heart, Lung and Blood Institute.

## ADDITIONAL RESEARCH INTERESTS OF THE PANIZZI LAB:

1) A separate NIH-funded project (5R44AI085840) has begun to develop new adjunctive therapies to treat acute endocarditis and other infections caused by *S. aureus*. This collaborative project between Auburn University, University of Mississippi and Lucigen Corp. has begun to investigate the efficacy and toxicity of novel anti-microbial drugs. To achieve this, researchers are making use of new *in vivo* probes to monitor the effectiveness of novel therapies and the impact that they have on bacterial infection.

2) The team also aims to develop a therapy for ischaemic injury caused by underlying atherosclerosis and dysfunction of an enzyme, myeloperoxidase, that results in a hypersensitive inflammatory response. The researchers hypothesise that by targeting this enzyme in treatment strategies it may be possible to subdue the immune response by reducing the activity of reactive oxygen and nitrogen species. Suppression of the immune response would enable proper wound healing in the context of atherosclerosis, thus improving patient outcomes for sufferers of this condition.

PETER PANIZZI


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# Probing the state of infection

Researchers from **Auburn University**, Alabama, USA are addressing the urgent need to develop more effective diagnostic and monitoring strategies for serious bacterial infections

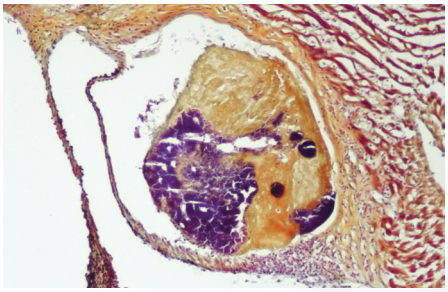
***STAPHYLOCOCCUS AUREUS*** **IS** a virulent bacteria that can cause a wide variety of infections and is the most frequently isolated bacterial pathogen from patients with hospital-acquired infections. Other examples of its pathologies include toxic shock syndrome, necrotising pneumonia, food poisoning and wound infections. Endocarditis is relatively rare in healthy individuals with no pre-existing heart-related problems, but because initial symptoms of the disease are flu-like (fever, headache and joint pain), diagnosis is often delayed and, for one in five patients, the condition is fatal. In adults, 30-40 per cent of endocarditis is caused by *S. aureus* infection and this figure increases to 40-50 per cent in cases involving neonates with congenital heart defects.

The disease trigger is often slight wear-and-tear to the heart valve resulting from repeated opening and closing. Typically, the body repairs this damage quickly, but if there any circulating bacteria present in the blood stream (a condition known as bacteraemia) the microbe can attach to the site of repair. *S. aureus* has the unique ability to wall itself off from host blood and tissue cells, avoiding detection and causing inflammation. Once the bacterium becomes attached to the damaged valve it rapidly multiplies and secretes factors to clot the blood, forming a wall that protects it from the host's immune system. These masses of fibrin, *S. aureus* and platelets can become large enough to obstruct or disrupt blood flow. Because of the virulence of these bacteria and their increasing resistance to antibiotic treatment, there is an urgent, clinical need to develop methods for the early and reliable diagnosis of *S. aureus*-based endocarditis. To facilitate this, research to develop a deeper understanding of the molecular mechanisms and regulatory processes involved in its pathology are of vital clinical importance. The development of novel, effective and non-invasive methods for the specific detection of *S. aureus in vivo* would be a significant clinical advancement.

With this task in mind, a team of researchers from Auburn University in Alabama, USA, led by Dr Peter Panizzi from the Harrison School of Pharmacy, is carrying out research to determine the mechanisms behind the growth of *S. aureus* endocarditic 'vegetations' to improve diagnosis of the disease. Their approach involves using *S. aureus*-specific identification strategies and techniques, making use of *in vivo* imaging technologies.

## SPECIFICITY OF VIRULENCE

Among staphylococcal species, *S. aureus* is unique in its ability to clot plasma. This makes it particularly virulent as it can lay down a protective fibrin layer, making it harder for the host's immune system to effectively clear the infection. *S. aureus* expresses two distinctive non-proteolytic prothrombin activators, namely staphylocoagulase and von Willebrand factor-binding protein that form an active bacterial cofactor-zymogen complex that specifically cleaves fibrinogen into insoluble fibrin. There is much unresolved about the reason *S. aureus* has this redundant system of prothrombin activators, but the imaging agents being developed by the Panizzi lab and collaborators will selectively target this unique type of activator. These prothrombin activators separate *S. aureus* from other microbes such as *S. epidermidis* because they enable it to produce the aforementioned vegetations *in vivo*. In addition to damaging heart valve tissue, the vegetations form tightly packed balls that become tethered to the site of valve damage by cables of fibrin tissue



Cross-section of a heart valve showing a tethered vegetation with bacteria in purple.

In this situation the disease can rapidly become life-threatening as the vegetations can detach from the valve tissue and pass into the circulatory system. When being transported in the blood, these clots can cause abscesses in distant tissue, or stroke if they pass into the blood supply of the brain.

## SPOTLIGHT ON INFECTION

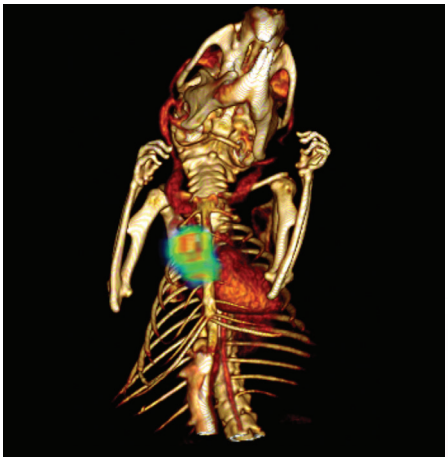
Existing methods of diagnosing bacterial endocarditis typically involve a series of transeasophageal echocardiograms, the results of which are often ambiguous and indeterminate. In humans the acute form of the disease can develop rapidly – within two weeks – but for effective diagnosis, the clinician must wait until the vegetation of bacteria, fibrin and platelets is large enough to be detected by the echocardiogram, by which time treatment can be less effective. To improve on this situation, Panizzi explains how his team is developing a multifunctional method: "We will use *S. aureus*-specific imaging to diagnose the causal bacterium, monitor the efficacy of therapy *in vivo* and assess clearance of the infection to minimise reoccurrence".

Advanced pre-clinical imaging techniques such as fluorescence molecular tomography (FMT) and positron emission tomography (PET) fused with computed tomography (FMT-CT or PET-CT, respectively) have been used successfully to visualise novel fluorescently- or chelator-labelled prothrombin markers in a mouse model of *S. aureus* endocarditis. Non-invasive imaging of these heart infections allowed for monitoring of antibiotic therapy and even relapse of the infection following insufficient antibiotic regimen. If these fluorescent and radiographic probes prove efficacious in a clinical setting, then the power of this tool means that, in a patient with endocarditis, it could be used to confirm the presence of *S. aureus* bacteria, visualise and analyse the exact location of the infection within the heart tissue and, over the course of the disease and treatment, monitor the spread of infection and its response.

## COMPLICATIONS

In order to test the efficacy of the fluorescent probes, the researchers have developed novel bioluminescent strains of staphylococcal bacteria. These genetically modified 'light-producing' microbes can be used to infect mouse models; fluorescent-imaging techniques can then be used to test the co-localisation of the bacterial-specific endocarditis probes with the bacterial infection during probe developmental. This is to ensure that when the probes are used in models with non-bioluminescent bacterial strains, they can correctly identify the strain of interest.

This process of genetic modification has not been without its challenges, as Panizzi explains: "Unlike Gram-negative bacteria such as *E. coli*, *S. aureus* has a very stringent defence mechanism known as restriction modification, which protects the pathogen from arrant incorporation of foreign DNA, such as our bioluminescent reporter, into its genome". Whilst this defence mechanism has made it significantly more complicated to produce the bioluminescent bacterial probes, research conducted as a consequence of this obstacle has led to a much better understanding of this regulatory mechanism and enabled cloning methodologies to be developed for carrying out the changes by circumventing the bacteria's restriction modification process.



FMT-CT image showing non-invasive detection of *S. aureus* endocarditis. Fluorescent probe incorporation into the growing vegetation is shown by heat map from green (less) to red (more).

## LOOKING FORWARD

With the successes the team has already experienced during this project, Panizzi has hopes that they can continue to deliver important contributions: "Further pre-clinical studies are needed to explore our pathogen-targeting fluorescence and radioactive probes in larger animals and initial human trials. Our continued development of new *S. aureus* probes and those that target other pathogens, like *Streptococcus pyogenes*, will allow us to delineate not only the location of the infections, but also the type of bacteria and bacterial load of the infection". With additional information about the pathobiology of the disease, it is hoped that this approach will enable earlier and more accurate diagnosis of endocarditis, be used to guide effective antibiotic treatment, and more accurately direct surgical intervention if a valve replacement is necessary.

Additionally, a more complete understanding of the molecular mechanisms that cause *S. aureus* infection will potentially give rise to alternative therapies. These could involve combating the infection by, for example, counteracting the generation of fibrin in the vegetations that effectively protect *S. aureus* from the host's immune response. Thus, going forward, the number of fatalities arising from late diagnosis and ineffective treatment of endocarditis could be reduced.

## INTELLIGENCE

### PATHOBIOLOGY OF *STAPHYLOCCOCUS AUREUS* ENDOCARDITIS

#### OBJECTIVES

The research focus of the Panizzi lab is to better understand how certain clinically important bacteria are able to cause human disease, and to use this knowledge against the microbe to detect specific sites of infections and identify the casual pathogens to allow for proper therapy.

#### PARTNERS

Center for Systems Biology at Massachusetts General Hospital (CSB-MGH) and Harvard University • National Center for Natural Products Research at The University of Mississippi (NCNPR-Ole Miss) • Lucigen Corporation

#### KEY COLLABORATORS

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**PETER PANIZZI, PHD** is an Assistant Professor at the Harrison School of Pharmacy at Auburn University. He received his doctorate from Vanderbilt University in May 2004 in the lab of Dr Paul E Bock. Panizzi completed additional training as a T32 postdoctoral fellow at the Center for Systems Biology at the Massachusetts General Hospital, 2007-10. He has co-authored over 30 high-impact articles and was awarded a prestigious National Institutes of Health (NIH) Pathway to Independence Grant (K99R00) from the National Heart Lung and Blood Institute before joining Auburn in autumn 2010.

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