4-2014

Effects of Antibiotics & Antifungals on Isolated Porcine Vasculature

Kathryn Young
Grand Valley State University

Effects of Antibiotics & Antifungals on Isolated Porcine Vasculature

Introduction:

Performing transplant surgeries, removing an organ from one patient and placing it in the body of another for physiological use, is now a commonplace procedure in western medicine. As of June 2013, there are 118,617 people on American waiting lists for kidneys, making it one of the most common transplant surgeries[9]. Though most of these surgeries have a high success rate when proper procedures have been followed, several articles have been published in relation to the systemic fungal and bacterial infections that occur in transplant patients[11,17]. To prevent against such infection as a result of transplant surgery, one might want to expose organs in transit from donor to recipient to some sort of antibacterial or antimicrobial agent. The use of low-dose antibiotics in buffer solutions appears to be somewhat common in basic cardiovascular research, making such a suggestion for isolated human organs a plausible proposal[12]. However, federal organ transport policies set forth by the Organ Procurement and Transplantation Network (part of the US Department of Health and Human Services) contain no transport guidelines requiring such a precaution in organ transport: in detailing the packaging of kidneys, only a sterile, watertight environment within a rigid container is requisite, along with appropriate labeling[10]. There also appears to be little in literature that assesses the effects such microbial compounds may have on isolated organ or vessel specimens: either in the context of organ transplant guidelines or basic study.

The research set forth in the following paper endeavored to conduct such an assessment, in order to add to the knowledge base surrounding isolated organs, both for the potential benefit of expanding organ transport guidelines and in improving the success

of *in vitro* physiological research. Porcine kidneys were decided upon as a suitable subject to study, due to the anatomical and physiologic similarities between pig and human renal systems[3]. The scope of the research was then narrowed to observing the effects of antibiotic compounds in isolated renal arteries, due to their integral role in the physiology of the organ[8], in hopes of laying the groundwork for future studies involving assessment of full organ function. Specifically, the research wished to compare the arteries' response to vasoactive compounds, in terms of increased or decreased function, dependant upon whether the vessel had been exposed to the appropriate antimicrobial.

Approximately seven weeks were spent isolating and characterizing various bacteria and fungi obtained from swab specimens of the porcine kidneys, taken upon arrival of the kidneys from a local slaughterhouse. Though no conclusive identification of a fungal species could be verified, characterization of the bacterial species was attained to the point of identifying an appropriate antibiotic, ampicillin, to which the prokaryotes were sensitive. This allowed for transition to the next phase of research, involving paired functional studies of arterial specimens exposed to either nutrient buffer, or both a concentration of antibiotic and nutrient buffer. While mounted on a force transducer, the changes in tension force exerted by the vessels was recorded, as the specimens were exposed to various doses of a vasoconstrictor and vasodilator; the recordings were used to determine an increase or decrease in functionality. Following data analysis, it was concluded that the concentration of ampicillin had a statistically significant inhibitory effect on both the constrictive and dilation responses of the specimens. Postulated reasons for this are discussed in the discussion section of this paper.

Methodology and Results:

Two porcine kidneys, from two distinct animals, were obtained from De Vries Meats Inc. of Coopersville, Michigan, following the slaughter of the pigs for meat processing. The kidneys were transported in a biohazard bag, on ice, to the research laboratory; bacterial and fungal specimens were collected upon arrival. Disposable cotton swabs were streaked across sample regions before being streaked onto either Brain-Heart Infusion agar, for bacterial growth, or Sabouraud's agar, for fungal growth, to be placed in an incubator for 48 hours at 37.5 degrees Celsius. Each of the 20 samples were organized and labeled according to the system in Table 1.

After incubation, the amount of microbial growth was qualitatively assessed visually; then, an inoculate of each distinct colony morphology was used to streak a Sab or BHI agar plate, respectively, in ordure to isolate each bacterial and fungal species into a pure culture (see Table 2). After 24-hour incubation of the bacterial samples and 48-hour incubation of fungal samples, the isolated culture plates were assessed for positive growth and photographed. Photographic evidence of the original plates, containing multiple species, was also obtained. Next, each isolated colony culture was confirmed as eukaryotic or prokaryotic, via observation of live, wet-mount microscopy.

Three of the seven isolated colony cultures on Sab agar were identified as eukaryotic species under 40x magnification: A1C, A2A, and A2B. Only A2A could be positively characterized as fungal, due to visualization of hyphae formation (see Figure 2D). The other four Sab isolates were characterized as prokaryotic growth; due to time and resource constraints, they were not further characterized for the project. The three eukaryotic cultures were further examined under low level microscopy, (see Figures 2A

and 2B for an example) in order to compare to descriptions of bloom morphology in fungal literature[14]. Hyphae formation pattern and bloom morphology allowed a tentative identification of A2A as belonging to the genus *Penicillium*[14]. In order to make a more definitive identification, a Colony DNA PCR procedure was run on all three eukaryotic samples and a control DNA sample, in hopes of eluting a characteristically sized genome[13,15]. The procedure was run four times without elution of any sample or control results. After the failure of each trial, first the enzymes preps, then the genetic primers, then the PCR cycle conditions themselves were altered, in hopes of producing product. The fifth PCR attempt, which utilized both a pre-mixed enzyme master mix prep and a piece-meal enzyme preparation, eluted product bands for both a 1:100 dilution of *Candida* control DNA and eukaryotic species A1C (See Figure 3). The A1C band elution past the 2100kb ladder indicated a larger genomic size than anticipated. Unfortunately, the PCR attempts were unable to produce a genomic product for specimen A2A, the suspected *Penicillium,* leaving its identification as only an educated guess. Due to time constraints, the colony PCR procedures were abandoned, in order to have time to move on to the vascular phase of research. Reasons why the PCR did not work are discussed in the discussion portion of this paper.

Eighteen of the twenty-one BHI isolated colony cultures were confirmed as prokaryotic via visualization under 1000x wet-mount microscopy for cell shape/morphology, and a Gram stain test[16] for further characterization done in duplicate. Three of the twenty-one cultures were not characterized for prokaryotic confirmation due to failure of the cultures to grow on fresh streak plates, after aging the originally isolated colony cultures for one week. No viable cell stock was available to perform a Gram stain on.

View Table 3 for full documentation of the characteristics for all eighteen bacterial species, and Figure 1A, 1B, and 1C for a prototypical example of full bacterial characterization.

Due to the large variety of specimens, though the majority fell into the category of Gram-negative staphylococci, it was decided to assess for sensitivity to broad spectrum antibiotics in the bacteria. Kirby-Bauer disc diffusion tests of all eighteen bacterial species were run, then observed for significant zones of inhibition after twenty-four hour incubation[16]. See Figure 1D for a visual depiction of a prototypical disc diffusion test. Four separate drugs, falling into one of three separate antibiotic classes were used in the tests, as follows: 30μg chloramphenicol c30, 30μg kanamycin, 10μg streptomycin, and 20μg ampicillin. More than 75% of the specimens exhibited extensive zones of growth inhibition that were large enough to indicate sensitivity to both chloramphenicol, a miscellaneous protein synthesis inhibitor[6,16], and ampicillin, a semisynthetic beta-lactam[5,16]. However, ampicillin was chosen as the most desirable drug to use in vascular trials due to its lower risk of toxicity in the arterial specimens[5,6,16]. The dosage of ampicillin to be used in the arterial studies was based on literature review of human IV dosing, assuming the slaughtered pigs from which the kidneys came was the size of a larger man: 120mg/5mL[2]. The characterization of the microflora and the approximation of a relevant antibiotic and dose allowed for transition to the next phase of research: paired arterial function studies.

For each day of paired vascular study, two fresh porcine kidneys were obtained from the local abattoir and transported to the lab in a cooler packed with ice. One arcuate arterial segment, measuring approximately two centimeters in length and three millimeters in diameter were dissected from each organ, each to serve as a single *n.* Two vascular rings, each measuring approximately 5mm in length, were then cut from each

segment and immediately mounted on force transducers in isolated organ baths (see figures 6 and 7). One of the paired rings was submerged in 12.5mL pure Krebs-Henseleit buffer solution, while the other was submerged in a Krebs solution containing 125mg/5mL ampicillin. Both were kept at 37°C, while 95% O_2 and 5% CO_2 gas was continuously bubbled into each bath. The vessels were then stretched to a passive tension of seven grams and subjected to a 45-minute equilibration period. Following equilibration, arteries were treated with cumulative doses of potassium chloride every five minutes (15, 30, and 45 mM) to observe constrictive response, then treated every five minutes with sodium nitroprusside to observe dilation responses (10^{-7}mM, $3x10^{-7}$mM, 10^{-6}mM, $3x10^{-6}$mM, 10^{-5}mM, $3x10^{-5}$mM, 10^{-4}mM). The amount of tension force exerted on the transducer by the arteries, via a free-hanging wire attached to a hook, was translated into the unit of grams and recorded in real-time by LabScribe2.0 software (see Figure 9). After an *n* of nine paired vessel trials was collected, the full LabScribe2.0 data results were analyzed in Microsoft Excel to reveal the average change in tension for both drugs (See Tables 4 and 5, respectively). The data was then condensed into graphical representation of the ampicillin vessels' average difference from maximum control reactivity in Figures 4 and 5. Finally, statistical significance of the results was determined utilizing SigmaStat ANOVA tests and two post-hoc analyses (see Figures 4 and 5).

Discussion:

The characterization of the microbial environment on the porcine kidneys was achieved with only limited success. Though a large number of prokaryotes, sampled from several different regions of the kidney, were easily characterized to the point of determining an effective antibiotic that inhibited their growth, no biologically relevant

antifungal compound was identified to use in the vascular phase of the research project. For the most part, this is due to the failure to identify any of the three eukaryotic morphologies in a meaningful way. Though general organism structure, in terms of hyphae formation and fungal bloom appearance, often aids in the directing of more stringent diagnostic tests, morphology comparison to literature isn't a valid method of positively identifying a physiologically relevant fungal species[1,14]. This was the reason for attempting to characterize the products of a genomic polymerase chain reaction (or PCR) of the eukaryotic samples, hoping to identify them via comparison to a pre-synthesized fungal genomic reference ladder used in other, relevant mycological work[13]. The general failure of the PCR procedures performed in the research presented here, appear to mostly be the result of a non-optimized procedure. Though problems with the activity of the *taq* enzyme present in a pre-made PCR 'Master Mix' was questioned initially, as was as the relevance of the forwards and reverse primer set chosen to aid in the amplification process, partial success of the PCR was achieved utilizing all of these potentially disruptive factors, following adjustment in the cycling temperatures of the procedure itself. In particular, the 'priming' or 'annealing' phase of the PCR cycle was dropped from fifty-four degrees Celsius to fifty-two degrees Celsius during the fifth attempt to obtain amplified fungal genomic product. The annealing phase of a polymerase chain reaction is critical, as it is the point at which the introduced synthetic primers non-convalently bond to the denatured target genome, in order to initiate a round of replication (or 'amplification') of the genetic material[15,16]. If the annealing temperature is too high, the primers will be unable to line up their base pairs with the 3' end of the target DNA, resulting in no association of the *taq* polymerase with the double stranded complex during the 'extension' phase of PCR and,

therefore, no increase in total genomic material. By obtaining at least one specimen and one positive control product, via two different preparations of *taq* enzyme, after adjusting the annealing temperature to a lower 52˚C, one may deduce that the cycle temperatures had not been previously optimized for the primers being used in this particular PCR procedure. Figure 3 illustrates the final, partially successful PCR gel produced during this research. In future attempts to characterize the fungal environment of porcine kidneys, which were discontinued in this body of work due to time constraints, further optimization of the PCR procedure would still be required in order to produce a full set of results. Ideally, one should be able to get positive elution from all three specimen genomes, as well as elution products for both the 1:10 and 1:100 positive control dilutions of pure fungal DNA samples. This successful PCR optimization would then have to at least be duplicated in order to be able to make a definitive identification the fungal species via PCR. One should also probably invest in a larger fungal genomic reference ladder as well, since both the specimen and control products that did elute during the research conducted here indicated genomes greater in size than that which can be measured by the ladder used (see Figure 3).

The impact of failing to identify the fungal species present in the biological samples and their antifungal counterpart be lessened though, by taking into account the relative scarcity of eukaryotic specimens isolated from the porcine kidneys. Only three specimens were obtained out of six samples from two different kidneys, and they were all conspicuously obtained from on the outer capsule of the organ, as opposed to the inner arterial or pelvic environment. This is in stark contrast to definite bacterial growth obtained from all sampled regions of both porcine kidneys, particularly the most biologically relevant environment: the inner arterial wall (see Table 2). This indicates that antifungal

compounds are at least a lower priority than antibiotics, in terms of preventing microbial contamination of renal arteries, if they are indeed necessary at all. The research therefore proceeded in achieving the primary goal of the microbiological phase of research: a compound was identified that was effective in inhibiting the majority of microbial growth obtained from porcine kidneys, in the form of the antibiotic ampicillin.

The mode of action for ampicillin is, at its core, identical to the mechanism used by all beta-lactam antibiotics. The cell wall of a prokaryote is made of polymerized, covalently bonded amino sugar subunits known as peptidoglycan The units polymerize using bi-functional penicillin-binding proteins (PBPs), whose transglycosidase sites forms new beta 1,4 linkages between adjacent molecules, until great strands of the polymerized peptidoglycan wrap their way around the entire prokaryotic cell. Furthermore, 'interbridges' are formed between covalent bonds of adjacent amino acid residues on the polymer subunits of Gram-negative bacterial cell walls, using the transpeptidase activity of PBPs to catalyze the reaction[18]. As one can guess by the protein name, all penicillin/beta-lactam class antibiotics, including ampicillin, bind irreversibly to PBPs and, therefore, inhibit the polymerization and interbridge formation of the bacterial cell wall. Due to loss of structural integrity, the bacterial cell is no longer able to keep up with osmotic regulation and eventually lyse due to an unregulated hypotonic state[4,16,18]. Ampicillin's particular strength lies in its synthetically generated side-chain, next to the nuclear rings, which allows it to pass through the outer membrane transport system of a wide variety of Gram-negative bacteria, therefore gaining access to their well-guarded peptidoglycan chains[4]. This ability significantly widens the variety of prokaryotic species that ampicillin can affect, making it an efficient, bactericidal broad-spectrum antibiotic. In contrast, many organically

synthesized beta-lactams, such as Penicillin G, have side chains that are not selectable for transport across a Gram-negative membrane[16,4].

The successful inhibition of bacterial growth by ampicillin was initially somewhat surprising, due to the fact that the cursory identification of fungal specimen A2A was thought to belong to the genus *Penicillium*, which is well known for producing beta-lactam antibiotics in the same class as ampicillin[16,18]. However, because that identification could not be verified, and the synthetically altered nature of the ampicillin compound itself would result in a variation of population sensitivity from organically derived beta-lactams[16,18], the results of Kirby-Bauer disc diffusion test were still plausible within the scope of the project.

The decision to assess the effect of ampicillin in renal arteries instead of chloramphenicol, the other broad-spectrum antibiotic to which many specimens were also sensitive, is based in its antibiotic mode of action. Chloramphenicol inhibits the function of the 50s prokaryotic ribosomal subunit, preventing amino acids fro being liked to growing polypeptides, therefore effectively halting the majority of protein synthesis within a bacterial cell[6,16]. The similarities that exist between eukaryotic and prokaryotic ribosomes, both of which are necessary to their respective cell's survival, are contributing factors in the documented high incidence rate of adverse reactions among patients treated with chloramphenicol[6]. As there is no peptidoglycan analogue present in eukaryotic cells for the ampicillin to act on, unlike chloramphenicol, it was determined that the risk of cytotoxicity would be less while using the beta-lactam antibiotic in mammal tissue[5,6]. This therefore made ampicillin the theoretically less cytotoxic and more appropriate antibiotic to use for the next phase of the research project.

Unexpectedly, though there is no eukaryotic cell equivalent to the target molecule of ampicillin, exposure of the porcine renal arteries to the antibiotic had a statistically significant inhibitory effect on the vessels' reactivity to potassium chloride and sodium nitroprusside. This is evidenced by the data present in Tables and Figure pairs 4 and 5. It should also be noted that, though there was significant decrease in response, all blood vessels effectively survived (to some degree) if they were exposed to ampicillin. This confers speculation that, through optimization of the experimental procedures and exposure, the negative effect on the vessels may be eliminated.

Though it cannot be determined with certainty without further study, there is more than one possible mechanism that may be generating the negative effect seen in the results of the vessel trials. As there is no eukaryotic analogue for the antibiotic to target within the human cell, there is only a slight chance of direct cytotoxic effects inflicted upon the isolated vascular tissue by the antibiotic[5,16,18]. However, there is a possibility of the compound interacting with molecules outside of the arterial tissue; for example the vasoactive compounds used to elicit vasoconstrictive and dilation responses from the blood vessels. After examination both of the drug interaction literature[2,5] and the experimental results however, it appears this possible mechanism for inhibition is invalid. For example, the expected sinusoidal trend of increasing vasodilation in response to logarithmically increasing doses of sodium nitroprusside, is generated by the average difference in tension between control and ampicillin-exposed vessels in Table/Figure 5. It is as if the antibiotic has a blanket suppressive effect on the response of the vessel that is indifferent to the ever-increasing dosage. If there was a reactivity between the vasodilator and ampicillin, one would have expected a more depressed sinusoidal shape or an absence of one, due to the

overwhelming greater concentration of ampicillin in the vessel environment (25mg/1.0mL) in comparison to the nitroprusside present (10^{-4} mM at its highest concentration). If interaction had occurred, the ampicillin would have consumed the vasodilator, instead of allowing it to affect the vascular tissue. The drug interaction simulators in two accredited clinician databases also produced no evidence of any interaction between ampicillin and either potassium chloride or sodium nitroprusside[2,5].

One possible cause of the inhibitory effect created by ampicillin in porcine renal arteries, is a significant change in the aqueous pH of the arterial environment, due to the antibiotic's presence. The carboxylic acid residue and many electronegative atoms in the ampicillin molecule itself, render it a fairly acidic molecule[5]. If added in high enough concentration, it is possible that ampicillin could drop the pH of the Krebs solution below that of homeostatic levels, creating an inhibitory affect on the vessel by way of inducing acidosis. If future work with this research is to be pursued, assessment of the effect that ampicillin's addition to Krebs solution has on pH should be documented, in order to either rule it out as a possible cause for decreased reaction, or to the issue.

Another, more probable, cause would be that the chosen concentration at which the vessels were exposed to did not take into account vital physiological variables, resulting in the exposure of vessel to extremely high levels of ampicillin. An extreme dosage of any compound, regardless of its mostly nonreactive interactions with eukaryotic cells, can induce a cytopathic effect. The concentration of ampicillin used in the experimental arterial rings was, as stated before, 125mg/5mL or 25mg/1.0mL. This value was obtained from Epocrates Online clinician database, as the lowest IV concentration or dose given to the average adult[2]. Assuming the pigs from which the kidneys were gathered would be

comparable in body mass and size to the average adult human, the concentration was used as it was attained, without further manipulation. However, upon re-evaluation and deeper reading into the other clinician databases, such as UpToDate, it appears that the information taken down as a low-end intravenously administered dose of the drug, was actually the low-end concentration of standardized IV ampicillin drip bags[2,5,]. It was not an adjusted dose for the blood volume dilution and real-time concentration that would be experienced by an average adult patient, let alone the concentration that be present in renal circulation in particular[5]. In fact, according to the new literature source, which is backed up by a length list of yet more peer-reviews secondary sources, the actual IV ampicillin dose range for an average adult, over a 24-gour period, is only between 100mg (divided into fractional doses every six hours) and 12g (divided into fractional doses every four hours). Taking into account that the average 70kg human male has an effective circulating blood volume of 700mL[8], this would mean the maximum concentration of ampicillin in the arterial blood at any given time would be 5.71g/1.00mL concentration: five times less than the dose experimental arteries were exposed to during the research presented here.

There are both advantages and disadvantages to this hindsight discovery of the inordinately high dose exposure the porcine arteries were subjected to. Unfortunately, it renders this body of data as being quite physiologically irrelevant, in terms of contributing to larger pools of knowledge in isolated organ studies closely related to human organ transport. However, it is beneficial to know the limits to which a potent antibiotic can be used without actually causing vascular death in an isolated arterial ring. Because the antibiotic concentration was exponentially higher than maximum recommended daily

dose, it would actually unexpected that the experimental vessels survived at all. That they did is a testament to the low eukaryotic toxicity of ampicillin.

Outside of realizing the extreme upper limits of antibiotic doses in renal vessels, the results presented here provide an easily-manipulated procedural framework to conduct more physiologically relevant research, as many of the microbial conclusions drawn in the paper appear to be sound. Further experiments could be conducted, utilizing ampicillin concentrations very similar to those experienced by renal vasculature *in vivo*, in order to assess the possible benefits of ampicillin in isolated vasculature. Continuing to utilize the paired vascular ring method would help control for variability between different kidneys and different specimen animals. One could even conduct pair studies to compare different concentration of ampicillin exposure directly to one another. From there, one may assess other potential variables of benefits or drawbacks. For example, would exposure to ampicillin allow for longer, or more rigorous vasoactive studies? Would antibiotic addition to HEPES preservative solution result in a longer shelf-life for isolated vasculature? These would be the last expected steps and evaluations in determining the optimal concentration of ampicillin to use in basic vascular research, and its overall beneficial or detrimental effects. Such conclusions could be publishable in basic physiological research periodicals, particularly those who focus on cardiovascular research.

Once that optimization has been achieved, assuming that a significant benefit has been documented in using ampicillin, the research could expand farther. By extrapolating the optimized concentration, one could conduct paired studies that assessed full isolated renal function in the presence or absence of penicillin. A set of two kidneys, both from the same animal, could be suspended in either nutrient Krebs solution or Krebs and ampicillin, then

monitored in terms of increased or decreased function by means of measuring 'urine' output. These fully isolated organ studies would, of course, also require several levels of procedural optimization as well. However, assuming such optimization could be achieved in the laboratory, and a significant benefit was documented in terms of relevant variables, such as prolonged shelf life while maintaining average reactivity to pharmaceuticals, the body of research, whose foundations are set forth in this paper, could easily become relevant and publishable in upper level physiological journals, and eventually, published work that deals directly with human organ transplantation procedures.

Selected References and Bibliography:

1. Campbell CK, Johnson EM, Warnock DW. *Identification of Pathogenic Fungi.* New Jersey: Wiley, 2013.
2. Epocrates. Ampicillin (generic). In: Epocrates Online, AthenaHealth, Watertown, MA. (Accessed March and April, 2014).
3. Heussner AH, Dietrich DR. Primary porcine proximal tubular cells as an alternative to human primary renal cells in vitro: an initial characterization. BMC Cell Biology, (2013) 14:55. doi:10.1186/1471-2121-14-55
4. Koetting M, et al. Hypothermic reconditioning after cold storage improves postischemic graft function in isolated porcine kidneys. Transplant International [Online]. GVSU library databases. http://onlinelibrary.wiley.com/store/10.1111/j.1432-2277.2009.01014.x/asset/j.1432-2277.2009.01014.x.pdf?v=1&t=hnc2luvh&s=3ace0c09990a06fcae6de47c112f283f9d74f070. [2013].
5. Lexicomp . Ampicillin: Drug Information. In: UpToDate, Post TW (Ed), UpToDate, Waltham MA. (Accessed April 16, 2014).
6. Lexicomp . Chloramphenicol: Drug Information. In: UpToDate, Post TW (Ed), UpToDate, Waltham MA. (Accessed April 16, 2014).
7. MacFaddin JF. *Biochemical tests for identification of medical bacteria*. Pennsylvania: Lippincott Willimas & Wilkins, 2000.

8. McCance KL, et al. *Pathophysiology: the Biologic Basis for Disease in Adults and Children*. Missouri: Mosby Inc, 2010.
9. National Kidney Foundation (NKF). Organ Donation and Transplantation Statistics [Online]. National Kidney Foundation. http://www.kidney.org/news/newsroom/factsheets/Organ-Donation-and-Transplantation-Stats.cfm. [2013]
10. Organ Procurement and Transplantation Network (OPTN). Policies and Bylaws Regarding Organ Procurement and Transplantation. A national clinical guideline [Online]. UD Department of Health and Human Services. http://optn.transplant.hrsa.gov/PoliciesandBylaws2/policies/pdfs/policy_172.pdf. [2013].
11. Pappas PG, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clinical Infectious Diseases [Online]. Pubmed.gov. http://www.ncbi.nlm.nih.gov/pubmed/20218876 [2013].
12. Qi W, Wei JX, I Dorairaj, Mahajan RP, Wilson VG. Evidence that a prostanoid produced by cyclo-oxygenase-2 enhances contractile responses of the porcine isolated coronary artery following exposure to lipopolysaccharide. British Journal of Anesthesia.(98) 3: 323-330, 2007
13. Sandhu GS, Kline BC, et al. Molecular probes for diagnosis of fungal infections. J. Clin. Microbiol. (1995) 33:11, 2913-2919.
14. Snell WH, Dick EA. *A Glossary in Mycology*. Harvard University Press. Cambridge, MA. 1971.
15. Snyder L, Champness W. *Molecular Genetics of Bacteria*. 3rd ed. ASM Press. Washington, DC. 2007.
16. Talaro KP, Chess B. *Foundations in Microbiology*. 8th ed. McGRaw-Hill. New York, NY. 2012.
17. Thomsen OF, Hansen HE. Bacteriuria and Renal Infection in Kidney-Transplant Patients. Acta Pathologica et Microbiologica Scandinavica [Online]. Pubmed.gov. http://www.ncbi.nlm.nih.gov/pubmed/343495 [2013].

18. White D, Drummond J, Fuqua C. The Physiology and Biochemistry of Prokaryotes. 4th ed. Oxford University Press. New York, NY. 2012.