JULIE ANDREWS

123 Pasteur Way, Norfolk, VA 23529

3/27/20

Editor
Current Pathogenesis
456 Salk Hall
Norfolk, VA 23529

Dear Editor:

I wish to submit the manuscript entitled “Pathogenesis of Uropathogenic *Escherichia coli*” for consideration by Current Pathogenesis Journal.
I confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

This manuscript describes the most updated background of Uropathogenic *E.coli* and the in depth pathogenesis of Uropathogenic *E.coli.* Based on current published papers, this manuscript also includes updated possible ways to prevent illnesses that come from it. This is significant because it is critical to understand the pathogenesis, lifestyle of pathogen and ability to colonize and infect Uropathogenic *E.coli* in order to further fight infections and possible fatal illnesses.
Our findings will allow your readers to understand the importance of pathogenesis of Uropathogenic *E.coli* and the depth of it, such as virulence factors. This will allow a better understanding of the pathogen and will serve as an updated way to prevent this bacteria from causing further illnesses or fatalities.

Thank you for your consideration of this manuscript.

Sincerely

Julie Andrews
Research Scholar
Department of Biomedical Sciences
Lovely Garden University

Dear Professor Blues,

It is much appreciated that you have given us the opportunity to revise, review and resubmit this manuscript. A special thank you goes to the reviewers and their suggestions. We have reviewed and made corrections. Specific responses for each suggestion are below. We look forward to the review of this improved submission.

Sincerely,
Julie Andrews

Reviewers 1 & 2 comments:

Below are the major issues with the manuscript:

1. The lines are not numbered.
Final paper- lines are not numbered.

2. There are no page numbers.
I have entered page numbers

3. There is no title or UIN number at the beginning of manuscript.
UIN is not to be on final paper, Title has been added.

4. Only the first figure has a description, but it is also missing a citation.
Updated description and citation for all images.

5. What is, “BEC”, “ec240”, “CUP-pili” and “P-pili”? These words were just mentioned, without giving a definite meaning or explanation.
Meaning and definition were included.

6. There should always be a space between the sentence and the citation. It was seen that there was sometimes a space, but in other instances, there was not.
All citations were fixed to be formatted correctly.

7. Make sure that for your citation that it is written as (“copied from [#]”), based on the assignment Instructions.
CItations fixed to show correct formatting.

8. Your figure legend along with the description and citation should look like (Figure 1. ... (copied from [#])).
Citations fixed to show correct formatting.

9. Make sure that there is a space between your sentence and in-text citation [ex. ... (#)]
Citations fixed to show correct formatting.

10. There is strange formatting (spacing) where Figure 2 is. This may be because of the figure, which would make more sense being center space and after the sentence, “Specialized epithelium such. as virginal epithelium is an acidic environment. This gives bacteria a good place behind inhibition and colonization(3). Figure 2 shows the infection process of the bladder and epithelium(3).”
This sentence is fixed to show correct formatting.

11. Make the subheading bolds, so it is clear to the reader that they are moving on to another section of the manuscript.
Subheading fixed to show correct formatting.

12. Label the unlabeled section before “Virulence factors” as “Introduction.”
Entered correct section heading

13. Add a “Discussion/Conclusions” section, to wrap everything up.
Entered correct section heading

14. For the sentence, “Symptoms for these infections are all similar. If infection is mainly in kidneys, also known as pyelonephritis, this is a more serious infection and symptoms include muscle aches, chills, fever and link pain(9)”, what is “link pain”. A brief explanation or a quick definition in parathesis next to the word would suffice.
This sentence explains the illness and describes the symptoms. Sentence has not been changed.

15. For the first paragraph under the section “Preventative and vaccine analysis”, it is too long. Find a way to split is up into two paragraphs, while making sure that the flow is sufficient.
Split into 2 paragraphs

16. For the section labeled “Preventative and vaccine analysis”, the section should be labeled as “Control and Eradication” instead.
Entered correct section heading

17. For the sentence, “The urinary tract is normally sterile, but when the urinary tract is breached by UPEC, its response is to trigger the inflammation response, this results in cytokines being produced as well an increase amount of neutrophil(7).”, stating that the urinary tract is sterile is not exactly true. There are good bacteria and bad bacteria, but it is not sterile. Doing more research on this would be beneficial, because the statement is too definite. What is mostly stated as being sterile in urine that is in bladder, but even that has been viewed as skeptical.
Sentence has been re-wore=ded to resolve misinformation.

18. Normally you don’t want to start a sentence with an abbreviation. What is normally done is making sure that you spell it out fully and include the abbreviation and then after that in your paragraph you can use the abbreviated version, as long as it is not the starter for a sentence.
Resolved all sections that started with abbreviation

19.The sentence, “Urinary Tract Infections still is one of the most common diseases across the globe(11).”, is not needed because you already talked about the statement previously.
Citation removed from this sentence

22. Overall, it is suggested that you use a grammar checker and/or carefully read through to make sure that sentences flow as you would like for them to and as they should, based on what is stated.
Reviewed and corrected flow of paper.

Uropathogenic Escherichia coli

Abstract

Uropathogenic *Escherichia coli* is the bacteria that is mainly known to be responsible for urinary tract infections otherwise known as UTIs.These are the commonest bacterial infections and play a huge part in the rise in healthcare costs. Though Uropathogenic *E. coli* or *UPEC* is the main cause of UTIs, it can also be linked to other health issues within the gastrointestinal system. *UPEC* strains are intelligent intracellular pathogens that are known for using their virulence factors to their ben*efit. UPEC* strains of *E.coli* have a variety of virulence factors, these virulence factors are used to attach and colonize within the host. Some of these factors include adhesions and toxins, though there are many virulence factors that could play a part. A majority of UTI pathogenesis research found on PubMed were research articles, but some were informational. Of 29 articles found regarding this topic. 17 of them were used and specifically researched virulence factors and the specific factors that give *UPEC* the ability to move from area to area as well as colonize and spread throughout that area. This ability allows the *UPEC* to cause further infection as well. The spread and lifestyle of *UPEC* has made it difficult to fight and prevent further infections. The host's immune system triggers response mechanisms that give protection and defense mechanisms as well as tools that are meant to keep the urinary tract healthy. With a recent increase of antibiotics as well as probiotics and the study of them, this has helped decrease the rise of infections. Though, there is still no "cure" only prevention from getting infection again. The virulence factor's ability to move and distribute as well as the lifestyle and survival abilities of *UPEC*, is a major key to understanding the pathogenesis of *UPEC*.

Introduction

Microorganisms play a major role in our everyday lives. Specific bacterias and pathogens can cause infections that are common among the population. *Escherichia coli* is a microorganism that causes many different infections due to its number of different strains.These strains consist of both pathotype strains and non pathotype, otherwise known as pathogenic *Escherichia coli* or non-pathogenic *Escherichia coli.* Pathogenic strains means that the bacteria is or is related to a pathogen (1). Pathogenesis is the study of how diseases develop as well as the actual mechanism of the infection. The differentiation between pathogenic and nonpathogenic is within the virulence factors, this

is how the microorganism sticks to the uroepithelial cell, how it invades and the manner of which it colonizes (2). *Uropathogenic Escherichia coli* (UPEC) is a main agent in causing the majority of urinary tract infections (UTIs) (3). Even with the host's defense system, *UPEC* can invade and colonize which can result in further infections (4).

Background

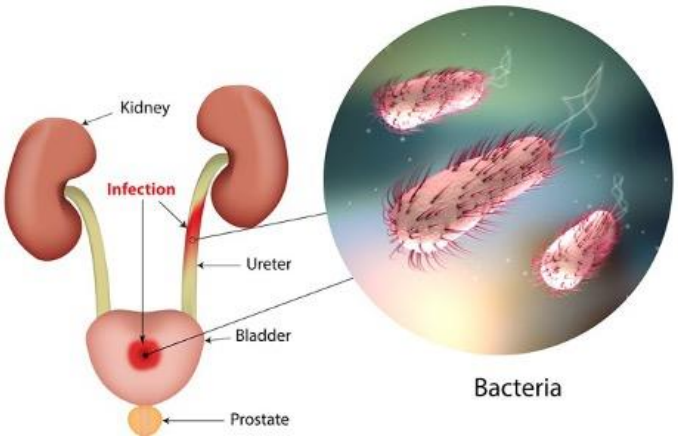
Uropathogenic Escherichia coli strains are smart intracellular pathogens, they are known for using their virulence factors to their benefit. Such as, taking control of the host's behavior and colonizing in a manner of aggression (5,6). Research shows that *UPEC* isolates itself into the human intestinal tract that is when the UTI develops (4). The research shows that the bacteria infecting and invading the host, often shows that it is rectal bacteria from that same host (7). *Uropathogenic Escherichia coli* is an incredibly diverse bacterial species, and has different phylogroups. The four main *UPEC* phylogroups are classified as A, B1, B2, and D (1,4). These have been identified and classified based upon the adhesions factors, the toxin secretions, pili/flagella, and other virulence factors. These are also classified based upon the genomic pathogenicity islands otherwise known as, PAI (1,3). *UPEC's* ability to transmit is not entirely clear. Studies also show that *UPEC* can directly be correlated through sexual transmission. It has been shown that *UPEC* strains taken from one partner, are a match to their sexual partner's fecal bacteria (6). The diversity of *UPEC* shows that it is related to *APEC*, which is a different strain of *Escherichia coli.* This strain is known as Avian pathogenic *Escherichia coli* (APEC) (5). *APEC* is the leading cause of septicemia as well as respiratory tract infections (9). It remains unclear as to how this bacteria makes way into the gut, though research shows that one possibility is by ingesting contaminated poultry, allowing these bacteria in and to act similar to *UPEC* (6,7,9). The sequencing of *APEC-01* with genomic analysis of other *APEC* bacteria, shows there is a genomic similarity with *UPEC*. The structures are similar within their strains (7).

Uropathogenic Escherichia coli, being the leading cause of UTIs has proven to have a major effect on the population and mainly, the effect is directly with women being the sensitivity to bacteria near vaginal canals (3,7). Women are more susceptible to urinary tract infections, this is because women have a shorter urethra and anatomically a closer region from urethra to anus, this makes it easier for the bacteria to enter (8,9) . At least 40% of women will develop at least one UTI during their lifetime (4). UTI's account for 70–95% of women's visits to emergency care and a large portion of medical costs (6).

Virulence factors

Uropathogenic *Escherichia coli*, otherwise known as *UPEC*, is the main cause of urinary tract infections and has many different strains and has a number of different virulence factors (10). These virulence factors are not only within the structure such as flagella or pili, but also secretions such as toxins (1,11). These virulence factors, and others such as iron uptake systems and resistance to serum, is the main difference between commensal *Escherichia coli* and *UPEC* (2,12). ***UPEC's*** adhesion ability is the most important factor to its pathogenicity (13). Adhesions directly correlate to the host and bacteria triggering signaling pathways, tissue supply and demand as well as invasion (15, 14).There is a number of different virulence factors, Type 1 fimbriae, remains unclear as to what part it plays in human pathology, yet it is shown to play a role with animal Urinary tract infections (16,17). P fimbriae is the virulence factor that is responsible for adhesion to the mucosal and tissue matrix as well as producing cytokines (15). The binding to epithelial and endothelial cell lines are done by S fimbriae and F1C fimbriae (13, 15).

Uropathogenic *Escherichia coli's* primary reservoir is within the human intestinal tract (9). Once *UPEC* is inside the tract, it will then begin to colonize the bladder (2). UPEC's binding to the tissues is a main factor of colonization. This assists in the bacteria's ability to fight against the flow of urine along with the invasion of the urothelial cells (5). The main adherence by *UPEC* are called pili/fimbriae. These are filamentous adhesive organelles (3). Invasion of the tissues are aggressively achieved through communication of the pious operons (13). This communication allows the expression the be switched, this also known as phase variation. This expression transfer allows distant pilus to be expressed. Which increases the adherence, affecting the overall invasion of these tissues (15). The bladder contains epithelial cells, these *UPEC* can form what is known as extracellular biofilms. These films are formed on the host's catheters and cells. Within the host's bladder epithelial cells, the films are also formed as intracellular biofilm communities (4). *UPEC* are then moved into being membrane-bound with acidic compartments, the bacteria begins to colonize, this is the start of cystitis, this is also known as lower UTI, with bacteria into the bladder (7,11). This bacteria can also move upwards into the kidneys and ureters. The infection of the kidneys and ureters is known as pyelonephritis, also known as upper UTI (7, 13).



(Figure 1)

Life cycle

Uropathogenic *Escherichia coli's* survival inside the urinary tract is based

on many things, but its ability to adhere is a main component to its survival (6). (**Figure 1** represents the urinary tract and where the bacteria infect it. (Copied from [1])). The urinary tract can be sensitive, it's vulnerability to become infected could be from many things, but genetic disorders and dysfunctions within the tract have been proven to have direct effects on the innate immune system's ability to respond to the invader as well as the invaders ability to survive (9,1). Urinary tract infections (UTIs) affect a majority of the population, a large proportion of the human population. About 150 million people worldwide develop UTI each year (3). Though this microorganism is mainly known for being the lead cause in urinary tract infections, it also has the ability to cause cystitis and other infections. *UPEC* cystitis has started being researched in depth on mice models to find more information out on this. Mice models are used as their bladder epithelial environment is similar to humans (14). Cystitis, if left untreated can cause kidney problems (12). If these infections are left untreated, the problems could get larger and more serious (17). The diseases this microorganism is able to cause is based on the virulence factors, one being urosepsis (3). Urosepsis is also known as pyelonephritis. If this is left untreated, this may result in renal failure or bacteremia (9).

Symptoms

Symptoms for these infections are all similar. If infection is mainly in kidneys, also known as pyelonephritis, this is a more serious infection and symptoms include muscle aches, chills, fever and link pain (9). Less serious, but still important infections such as cystitis, have symptoms such as burning when urinating, frequent urination, lower abdominal pain (10).

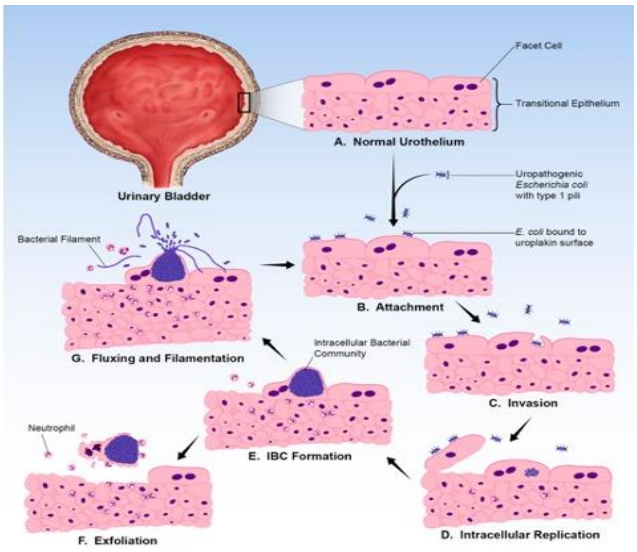
Host response

The urinary tract is is close to sterile from pathogens, there is bacteria that makes up the tract- but it is necessary, when the urinary tract is breached by *UPEC,* its response is to trigger inflammation, this results in cytokines being produced as well an increase amount of neutrophil (3,7). The bladder's epithelial cells that are infected begin to exfoliate and antimicrobial compounds as well as nitrogen and oxygen begin to generate the exfoliation of the infected bladder epithelial cells (9,3,10). The innate immune system begins to respond with PAMPS(pathogen-associated molecular patterns) or DAMPS(danger/damage-associated molecular patterns), these patterns are identified and recognized in epithelia and specialized cells by PRRs (Pattern recognition receptors) (15,1). Specialized epithelium such as virginal epithelium is acidic environments. This gives bacteria a good place behind inhibition and colonization (3).

Control and Eradications

The bladder's cells gives multiple protection and defense mechanisms And tools that are meant to keep the urinary tract healthy and sterile (1).The urinary tract is kept

healthy by the natural flow of urine, which allows any microbes to be flushed out, this also has antimicrobial molecules and effector immune cells that act as protection as well (9,13). Even with all these protection mechanisms, the Urinary Tract Infections still is one of the most common diseases across the globe. The bacteria enter the urinary tract and begin to infect and colonize. *UPEC's* ability to attach and colonize within the host's epithelial cells in the urinary tract, is the determining factor of its pathogenic ability and life span within the host (1,8). Once the cells begin to exfoliate from infection, the host begins to clear the bacteria by using the flow of urine (8). This process leaves the cells more apt to infection as they're more exposed (7). Research recently done with different pathotypes of *E. Coli* shows that *ABU,* also known as asymptomatic bacteria *E. coli,* act as a protection mechanism for the urinary tract by lining and protecting from *UPEC colonization. This is also known as a commensal relationship, where one organism assists another and they both benefit (1). (**Figure 2** shows the infection process of the bladder and epithelium(Copied from [3]))*



(Figure 2)

Preventative/Vaccine:

Uropathogenic *Escherichia coli* being the leading cause of urinary tract infection which in turn can lead to other diseases, can be prevented with certain approaches. Most women who have been infected once, are more likely to become infected again and have chronic urinary tract infections (10). These recurrent infections have been studied and shown that the *UPEC* changes the actual vaginal or periurethral microbiota and the preventative mechanisms to ensure the diseases that *UPEC* causes do not return, have been studied and proven that probiotics are able to reduce the *UPEC's* ability to adhere within the urinary tract (1,15). Within the urinary tract infections, it is likely that in women vaginal canals are to be infected as well and cause bacterial infections within the vaginal canal.

Research shows that lactobacilli have the ability to regulate NF-κB activity by stimulating the immune system's response which allows them to colonize and act as a defense against *UPEC* within the vagina (11). Another preventative mechanism that has been researched outside of antibiotics is the prevention of adhesion by inhibition. *UPEC* type 1 binds on the surface of urothelial cells, the terminal epitopes of high forms of sugars attached with uroplakin (11,7). By preventing the actual binding to BECs (Bladder

epithelial cells) and biofilm formation, it prevents colonization (9,13). This method is done using pilicides. Pilicides are derivatives of ring fused 2- pyridones, which act as a blocker for the formation or adherence of pili (1,10). This has been researched and proved to prevent close to 90% of adherence by type 1 *UPEC* and/or P-pili (12).P-Pili are a specific type of pilicide that have a distinct adhesive tip (3). Another specific pilicide is ec240, this pilicide is one of the most potent inhibitors, this was studied in depth and shown to decrease movement as well as un-regulate the pili within the Chaperone-usher pathway(CUP) (9,12,15). Other research for preventatives for *UPEC's* shows that estrogen therapy may play a part in the bacteria's ability to survive within the tract. Oral estrogen therapy showed to be ineffective in prevention of UTIs, but vaginal estrogen therapy showed to be effective in women who have gone through menopause (11). Effective vaccines for the diseases caused by *UPEC* are still being developed. Vaccines to prevent UTIs and other *UPEC* caused diseases are focused primarily on the specific factors within the bacteria *UPEC (1).* Vaccines have yet to be successful, but any potential antigens are focused on a permanent haunt of adhesion and potential AMPs or antimicrobial peptides that can permanently be effective within the urinary tract (14).

Discussion/ Conclusion

Uropathogenic *E. coli* has and uses many different virulence factors to colonize and infect the urinary tract (Figure 2). Pili are essential for the bacteria to be motile and move throughout the periurethral into the bladder, this is where *UPEC's* adhesion factors come into play and colonize the uroepithelium (16). Pili also can move *UPEC* through the ureters into the kidneys which can result in renal failure if no treatment is received. The host's response and defense mechanisms begin and exfoliation of the bladder's epithelial cell begins. This triggers the inflammatory response and the sequence of iron response. *UPEC's* ability to respond to these defense mechanisms is to produce different secretions that evade them being killed by the immune responses from the host. There are many virulence factors that are known and have been studied for *UPEC*, but the ultimate force of action has yet to be determined as there are still factors that are being found and researched. *UPEC* are pathogens that have the ability to alter their response based upon the host's responses and are able to not only colonize the human gastrointestinal tract but also cause disease within the urinary tract.

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