Qualitative Real-time PCR Analysis for the Presence of Mycoplasma and Ureaplasma Bacterial Species associated with Urinary Tract Infections

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Introduction and Objective

Common bacterial infections that cause UTIs are Escherichia coli, Klebsiella sp., Proteus sp., and sometimes Staphylococcus sp. and Streptococcus sp. These infections are identified through culturing techniques using Sheep’s Blood Agar (SBA) and MacConkey Agar (MAC) followed by chemistries. Though these bacteria are most commonly identified as the cause of urinary tract infections (UTI), many times a suspected UTI will yield negative culture results and be reported out as negative for growth to the provider. Fastidious microorganisms (such as Mycoplasma genitalium and Ureaplasma urealyticum) can be considered normal urethral and vaginal flora or a possible source of sexually transmitted infections. Not much is known about the role these bacteria play in common UTIs. Mycoplasma sp, Staphylococcus sp., Streptococcus sp., Ureaplasma urealyticum, and sometimes Staphylococcus sp. and Streptococcus sp. are most commonly identified as the cause of urinary tract infections. Using molecular techniques, we tested for the presence of M. genitalium, and U. urealyticum through a qualitative analysis. The Quagen DNA isolation kit was used for DNA isolation, and DNA concentration was determined using a nanodrop instrument. DNA concentrations >5μg were confirmed for presence using gel electrophoresis. Species specific Taq-man probes were used in real-time PCR to check for the presence of Mycoplasma species or Ureaplasma urealyticum. A statistical sensitivity and specificity analysis was performed along with a chi-square analysis for a comparison of observed vs. expected results.

Methods

Specimen Collection

Urine samples for suspected urinary tract infections (UTI) from the Student Wellness Center on TTU main campus (an entity of the TTUHSC system) were obtained after physician’s orders for each patient had been set up for analysis but prior to sample disposal. Specimens are ones with orders for urine cultures that either did or did not exhibit growth of bacteria under traditional culturing techniques on BSA and MAC agars. Each sample (n=37) was de-identified and either did or did not exhibit growth of bacteria under traditional culturing before sample disposal. Specimens are ones with orders for urine cultures that either did or did not exhibit growth of bacteria under traditional culturing techniques on BSA and MAC agars. Each sample (n=37) was de-identified and either did or did not exhibit growth of bacteria under traditional culturing before sample disposal.

Results

In order to determine if there is an association between symptomatic patients’ culture results, a chi-square test of independence was used. Because this analysis requires a minimum of five data points, Mycoplasma was excluded. Ureaplasma was not found in specimens that did not produce growth on traditional cultures or that were probably contaminated. Ureaplasma was however detected in seven urine specimens that did produce growth. The results indicate that there is a significant association between symptomatic patient culture results and the presence of Ureaplasma urealyticum being transmitted through sexual routes.

Discussion

The results show sufficient evidence that we can reject the null hypothesis for Ureaplasma urealyticum. There is an evident association between culture results and the presence of Ureaplasma urealyticum using molecular techniques; however, the correlation is not in the culture negative specimens as initially suspected. More specifically, the results show an association between the growth on bacterial plates with the presences of Ureaplasma. Results indicate that there is no clear correlation between Mycoplasma and UTI specimens. Therefore, we cannot reject the null hypothesis for this group. Further research is needed to explore the possibility of Ureaplasma urealyticum being transmitted through sexual routes.

Conclusion

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References