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MOLECULAR DETECTION OF 18S rRNA GENE OF ENTEROBIUS VERMICULARIS AND 16S rRNA GENE OF SOME BACTERIA FROM INFECTION FEMALE AT AL-KUT CITY

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Abstract:

This work took place in Wasit Province, Iraq, and involved 96 female urine samples from suspected Enterobius vermicularis parasitic infection patients between October 2020 and April 2021. and after that cultivated on blood and MacConkey agar for diagnosis with API staph, API 20E, and polymerase chain reaction (conventional PCR). The results related to the urine sample showed examined by PCR. The traditional PCR approach was used for detecting E. vermicularis on the basis of 18S ribosomal RNA gene and the result was 20 positive samples out of 96 asymptomatic pinworms. the results for urine culture samples have been 70 positive samples (72.91%) out of 96 belonging to various bacteria types. Following that, a PCR test was performed on 96 samples of the same urine samples to confirm the existence of bacteria, with 24 (25%) of the bacteria cultured on blood agar and MacConkey agar yielding positive results. During the research, it was discovered that the incidence rate in rural areas is more than 53 (50.88%), while in urban areas it is 43. (41.28%).

Keywords: PCR, Scotch tape, *Enterobius vermicularis*, Bacteria, Culture

Introduction

Globally, one of the main health issues is the intestinal parasite infection. The World Health Organization (WHO), states that 24% of the population in the world are experiencing intestinal parasites with major occurrence in poor nations (Norman *et al*., 2015). Environmental and climate conditions are directly impacting the intestinal parasites' spread, since they are heavily involved in the transmission and migration of parasites in nations not previously-inhabited through them. Thus, nematodes were of high importance for many researches with their diagnostic, epidemiological, preventive and pathological strategiess (Mabrook and Yahia, 2006). *E.vermicularis* is known as pinworms or seat worms belonging to the nematode helminthes and are specified as a major parasite all over the world)Kucik *et al*., 2004). In addition, the pinworm has been characterized via its creamy white color and the male's length is between (2 and 5) mm, while the female's length is between (8 and 13)mm, also its major analgesic is in the intestine's ileodermal region, yet it is moving in all the gastrointestinal tract parts from stomach to the anus, in which the female adult pinworm starts to move at night to rectum for laying the eggs in the anus, in which the eggs develop to the infection stage in some hours because of the body temperature. Those eggs were specified by being capable for staying in a humid environment for (2-3) weeks, yet the majority of injuries happen throughout the eggs' transfer from the anus to the mouth via a hand that is contaminated with eggs, and such condition is common (majorly in children) Eckert *et al*., 2005. Furthermore, the major approach for the pinworm parasite' transmission is via direct infection between uninfected and infected people (Patsantara *et al*., 2015).

Material/Subjects/Patients and methods

Sample Collection:

A total of 96 urine have been obtained from women aged 8 to 45 years old for this work. The research has been carried out between October 2020 and April 2021 at Al Kut Hospital for Women and Children, Al Karama Teaching Hospital, Al Numaniyah General Hospital and its health centers, Al Zahra Teaching Hospital, as well as samples from middle and primary schools for girls in Al-Kut city. Those samples have been collected in sterile plastic containers to collect the samples of urine first thing in the morning.

The data of patients were collected through a survey which contains the age, date of collection and a question regarding the existence of a few disease symptoms like itching in the anus throughout the night and infections in urinary tract, weight loss, abdominal pain, loss of appetite or the presence of any other symptoms, and asked if the children washed their hands following leaving the toilet, typically sucked it with their fingers, and lastly an inquiry regarding the mother's educational level.

The samples have been divided into 2 parts, the first one is investigated in two hours or less, while the approach used to collect urine samples is to be stored in sterile collection tubes and after that transferred to the laboratory for diagnosis and culture, the culture has been on Macconkey agar and blood agar through planning dishes and incubating them for 18-24 hours at for diagnosing the bacterial growth it has an Analytical Profile Index (API) and the sample of the second part is kept at -20 ° C for PCR examination.

Samples Analysis

- **Gulture media**
- **API20 and API staph**
- **Molecular Diagnosis**

AccuPrep Stool DNA Extraction Kit, Bioneer, Korea, has been utilized for extracting the genomic DNA from stool samples, which was done according to the company's instructions: A stool sample of (200 mg) was transferred into a clean centrifuge tube, followed by the addition of 20µl of proteinase K, 400µl of stool decomposition solution (SL), and 10020µl of isopropanol

while mixing the samples, and finally 500µl of W2 buffer (ethanol) for each column. The dried DNA filter column has been transferred into a clean 1.5µl micro-centrifuge tube, and 50µl of pre-heated rinse solution was poured into the centre of the column matrix. The tubes were after that left standing for at least five mins to allow the buffer to be absorbed by matrix. The PCR product was then examined using agarose gel electrophoresis after centrifugation at 10,000rpm for 30 sec. to remove purified DNA.

Results:

96 urine samples were taken, and the results of the examination were in three sections. The first stage was PCR test examined for 18S ribosomal RNA E. vermicularis, and the result was 20 positive samples, while the second section was 24 positive samples for PCR test examined for 16S RNA bacteria. The third part is the culture media, where the urine was cultured on blood agar and MacConkey agar and then it was diagnosed on Api 20 and Api Staph , and the results showed in the table (1) and (2).

Discussion:

The urine sample results showed showed 20 positive samples (20.83%) of the total 96 a result in the examined samples when studied through PCR. The present findings were almost identical to those of (AL-Jaf, 2018), whose findings were nearly (81.5%). In addition, according to Ozcelik *etal*., (2009), the prevalence of E. vermicularis in Turkish orphanages is (80%). According to Rahi (2010), the incidence of pinworms in primary school in Wasit province has increased (65.3%). According to Al – Qadhi *etal*., (2011), the percentage of E. vermicularis infection in Baghdad orphanages has been 84.3%. Hussein, (2015) was reported in the province of Najaf (83.9%).

Unlike other results, the current study's result is high, and the percentage was higher compared to that indicated in Basra (Rhadi, 1994). (35.3%). Ganem recorded 31.2% in Kirkuk (1996). Al-Khazragy (1998), in Baghdad, recorded (34.20). Al-Izzi (1998) was recorded in Mosul (15%). Kadi & Amin reported a 29.5% infectivity rate in Sulaymania /Iraq (1998). In Baghdad, (Al-Mosawi, 2003) reported (41%). In Erbil, which was recorded by (29.8%) (Kader and Salman, 2011). The recorded percentage in Baghdad was (49.90%) (Al-Najar, 1999). The percentage in Alkhalis, Diyala was 9.4% as indicated by Al-Khalis by Al-Bayaty (2000).

In Baghdad, 13.2% of infected children with enterobiasis were found (Saeil, 2005). Makhlouf et al., (1994), reported on other epidemiological studies in Egyptian orphanages (60%).

In the case of urine culture samples, 70 of the 96 samples belonged to different bacteria (E. coli, S. aureus and Klebsiella) cultured in blood and McConkey agar as shown in Tables (1) and (2) above, and then assayed by 16S RNA method The results were 24 (25%). And comparing it with the results of the PCR test for pinworms, which were 20 positive out of 96 samples, we can point out from this that there is a link between pinworms and urinary tract infection. This research agrees with U. Z., Ertan et al (1999) In girls, our findings revealed a link between urinary tract infection and Enterobius vermicularis. Pinworms were found in considerably higher numbers ($p = 0.030$) in girls with UTI, particularly the ones over the age of six ($P = 0.012$).

An increase in the number of girls with pinworm infection and urinary tract infection, possibly as a result of worm depletion in the urethra and bladder, which could be the cause of urinary tract infection (Simon RD, 1974). Nematodes appear to only be able to enter the urinary tract through the urethra. Female worms typically die or return to their right intestinal habitat through the anus after laying eggs at night. They can get lost and enter the vaginal canal, after that ascend to the reproductive system till, they reach the peritoneal cavity via the uterine tubes' cavity (Smolyakov et al., 2003).

Nervousness, nightmares, insomnia, and convulsions are symptoms of the pinworm parasite infection. The subsequent migration of worms to the vagina, uterus, or fallopian tube can also be seen in mucous secretions, yet a large proportion of pinworm infection was reported with no symptoms (Herrstrom et al., 1997).

The highest infection (50.88%) was found in patients who lived in rural areas, whereas the lowest infection (41.28%) was found in patients who lived in urban areas. Rahi (2010) in Wasit province and Kadir and Amin (2011) in Sulimaniya province, Iraq, both came up with similar results. People who live in rural areas are more likely to be infected than those who live in urban areas (Herrstrom et al., 2001). Infection rates differed from one region to the next, possibly due to people's instructive levels, crowding indexes, poorly maintained areas and poor sanitary disposal in areas where infection rates were high (Kader and Amin, 2011).

Conclusion:

Among the other intestinal parasites, the pinworm parasite is thought to be the most common. The study's findings revealed an increase in the percentage of people infected with pinworm parasites, which was influenced by a variety of factors including age, geographical distribution, economic status, gender, and parental education level. The findings of the study revealed that there is a link between pinworm infection and urinary tract infection.

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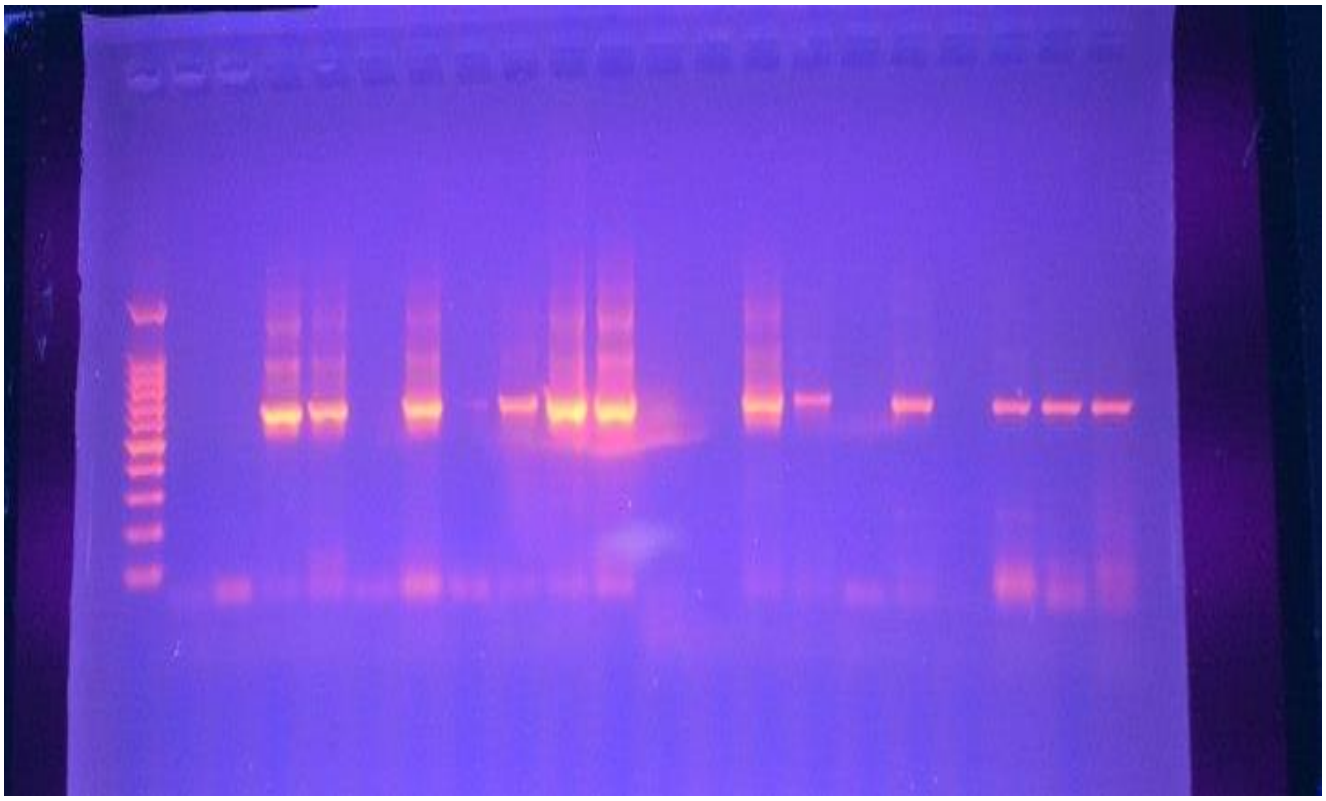
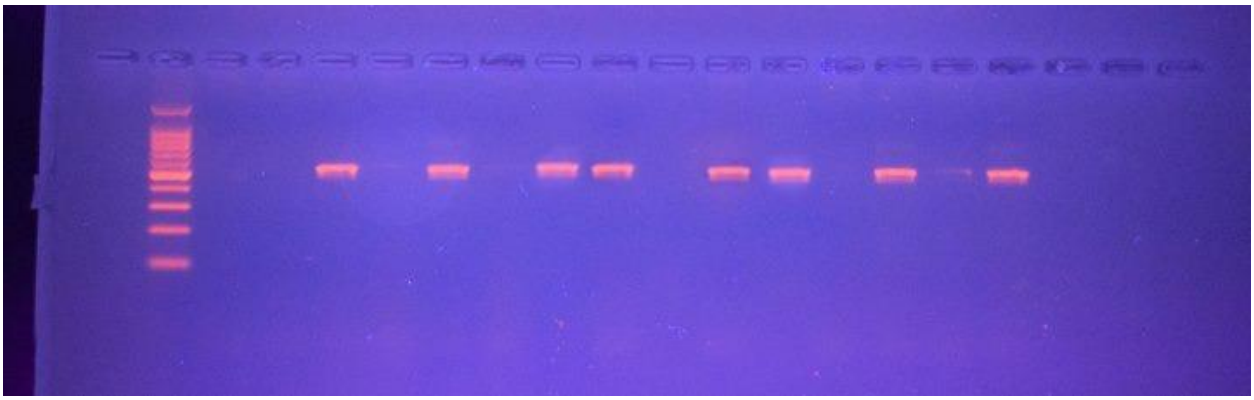
Bacteria	*E.coli*	*S.aureus*	*Klebsiella*	Total
No.	11	9	4	24
%	19.64%	16.07%	7.14%	42.85%

Table (1): Results of PCR Test for bacterial.

Table (2). Results of bacterial culture

Bacteria	*E.coli*	*S.aureus*	*Klebsiella*	No growth	Total
No.	35	25	10	26	96
%	36%	26 %	10%	27%	100%


2000 bp
500 bp
100 bp


1500 bp
500 bp
100 bp

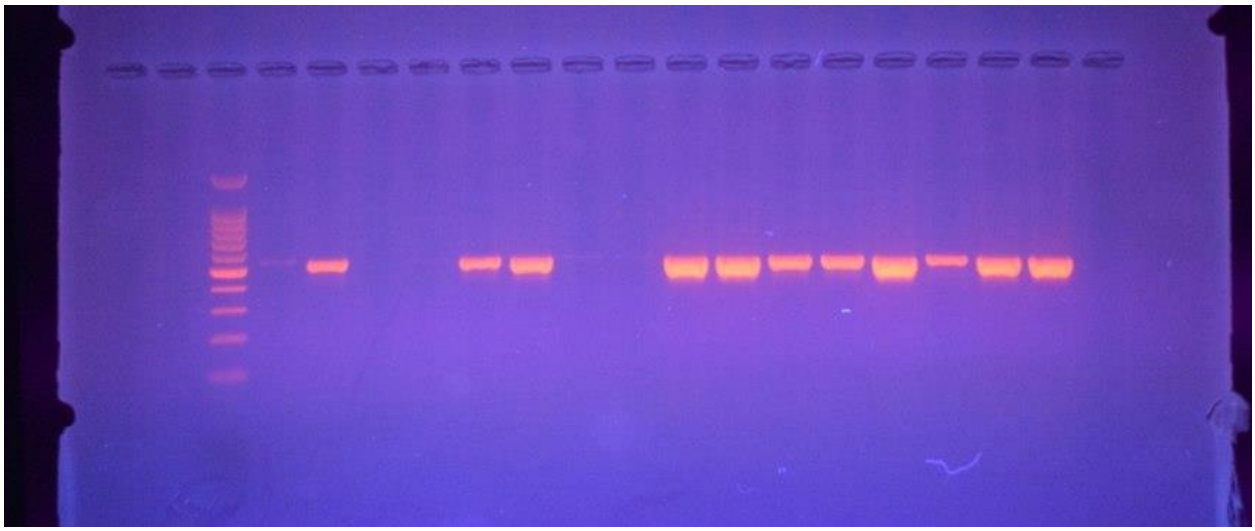


Figure1: Agarose gel electrophoresis image that shown the Conventional PCR, Where M: Marker (1500-100bp). A- product of mitochondrial RNA ribosome 18S gene for *Enterobius vermicular*, B- RNA ribosome 16S gene for *E. coli* , C- RNA ribosome 16S gene for *Staphylococcus aureus*.