

Isolation and identification of *Burkholderia cepacia* from aborted women with study of virulence factors genes

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Abstract

Abortion is the most common complication during pregnancy in women, with a rate of 15-20% among pregnant women approximately 80% of spontaneous abortions occur in the first trimester. A descriptive and controlled study was performed on women who had spontaneous abortions to identify risk factors for spontaneous abortion. They were admitted to Al-Zahra Teaching Hospital (maternity ward and emergency ward) from method of work Urine samples and vaginal swabs were taken from aborted women and pregnant women from, Al-Furat Hospital, and Al- Hassan al askary Clinic in Al-Najaf Al-ashraf this study involved (200) samples were collected from women with abortion and pregnant, all subjects were within 13–46 years of age. The sample include vaginal swabs from miscarriage female during the period from October 2022 to February 2023. The results showed *Burkholderia* percentage of isolated bacteria from aborted women was *Burkholderia cepacia* (31%). and the following genes *gyrB/trpB/parC/recA/gyrA/phaC/gltB/atpD* were detected. Where the percentage of each of these genes appeared as follows. *gyrB* gene 8(66.6%), *trpB* gene 11(91.6%), *parC* gene 12(100%), *recA* gene 12 (100%) *gyrA* gene 6(50%), *phaC* 11(91.6), *gltB* gene 10(83.3), *atpD* gene 8(66.6).

Introduction

Abortion is the process of ending a pregnancy by removing a baby or embryo from the uterus before it has a chance to survive. One of society's most significant medical issues is abortion. In addition to its impact on low-income populations, it also contributes to family breakdown. Three successive pregnancy losses or more are considered recurrent miscarriages (1). RPL is a complicated condition whose origins have been linked to a variety of variables, such as chromosomal abnormalities, immunological and immunogenic, endocrinological, DNA fragmentation in sperm, impairment of the endometrium's biosensor function, and lifestyle factors. 4. For many couples, routine studies are the norm, and in about 50% of instances, the cause of RPL is judged to be "unexplained" (2). Addition Maternal age >35 years or paternal age >40 years, maternal overweight, African-American ethnic group, smoking, coffee or alcohol consumption, exposure to ionizing radiation or pesticides, maternal stress, a history of prior SM or elective termination of pregnancy are some risk factors that have been shown to contribute to the development of SM (3). Bacterial genital tract infection during pregnancy can have an impact on both the mother and the fetus. An example of this is when *Streptococcus* spp. It can lead to severe pneumonia and meningitis in newborns, both of which frequently result in sepsis (4).

Generally widespread in nature, *Pantoea* spp. has been isolated from a variety of biological niches, including plants, water, soil, insects, and animals, but is still thought to be a rare opportunistic pathogen to humans (5). Following that, *Pantoea* species have been increasingly isolated from clinical sources and have been linked to meningitis, osteomyelitis, septicemia, urinary tract infections, and bloodstream infections (6). A gram-negative bacterium known as *Pantoea agglomerans*, formerly known as *Enterobacter agglomerans*, can be isolated from soil and plants (7). Despite usually living in symbiosis with vegetables, this bacteria can cause serious plant diseases. *P. agglomerans* infections have only seldom been reported in other vertebrate animals, but have been isolated from cases of septicemia, peritonitis, arthritis, and abscesses in humans. These infections are typically caused by traumatic injuries connected to plant or wood material (7). urinary tract

infection (UTI) following kidney transplantation is the most typical infectious complication. Burkholderia cepacia recurrent urinary tract infection is an uncommon finding. In the event of recurring urinary tract infections (UTI) following kidney transplant, a thorough anatomical examination is crucial. Important risk factors were neurogenic urinary bladder and vesico ureteric reflux (VUR) (8). with Burkholderia cepacia is an uncommon discovery. In c, a thorough anatomical assessment is crucial. In the hospital setting, Burkholderia cepacia is a common gram-negative, aerobic, non-fermentative bacterium. Additionally, it is an opportunistic pathogen in immunocompromised cystic fibrosis or chronic granulomatosis patients (9). Burkholderia-related nosocomial outbreaks of urinary tract infections (UTI), blood infections, and lung infections have been common in recent years. ward water sources, oral drink, ultrasonography gel, skin disinfectant, and mouthwash that have been contaminated with cepacia (10). Small organic molecules produced by Bukholderia spp. bacteria, commonly called as natural products, enable these microorganisms to adapt and coexist in a range of ecological contexts (11).

Methods

Sample Collection

This study involved (200) samples were collected from women with abortion and pregnant (one hundred from abortion women and one hundred from pregnant wome), all subjects were within 13–46 years of age. the sample include vaginal swabs from miscarriage female Al-zahraa Hospital and Al- furat Hospital during the period from (octoer 2022 to february2023). The swabs of vaginal discharge were obtained from woman by brushing a swab across the vaginal wall and urine sample the used swab was placed into Aims’ transport media to be used for the bacteriological diagnosis . were collected and sent to the lab, where they were streaked on MacConkey agar, a blood agar.

Bacterial isolation

The acquired sample should then be incubated at 37°C for 24 hours on MacConkey Agar, Blood Agar, and (MSA)Mannitol Salt Agar.The isolated colonies were then cleaned and cultured. nutritional agar.The isolates' pure colonies were chosen using sheep blood agar(12).Visual and genomic traits were used to identify the bacterial isolates. Features of biochemistry are described in Bergey's Manual of Systematic Biochemistry. Bacteriology. Gram staining was utilized to evaluate the isolate bacteria's cellular structure, and the VITEK2 compact system was employed to determine their identity (13).

Identification of Bacterial Isolates: Complete identification of each bacterial isolates was based on a cultural examination, morphological examination, and biochemical characterization

Results and Discussion

The study consisted of 200 samples, divided into two groups, the first group of women who suffer from abortion recurrent considered patients with about 100 samples (50%), and the second group of healthy women consider control about 100 samples (50%). The included 100 women with abortion with age range (10-50 years), the study showed that 56 of 100 were within the age group 20-30 years and 7 were 10-20 years with mean age (28.5 years) and 28.83 of cases were rural.and 28.21 of cases were urban as seen in Table 1.

Table 1: General characteristics of women abortion (n:100)

Characteristics	Aborted women (n:100(
10-20 years	7
20-30	56
30-40	32
40-50	5

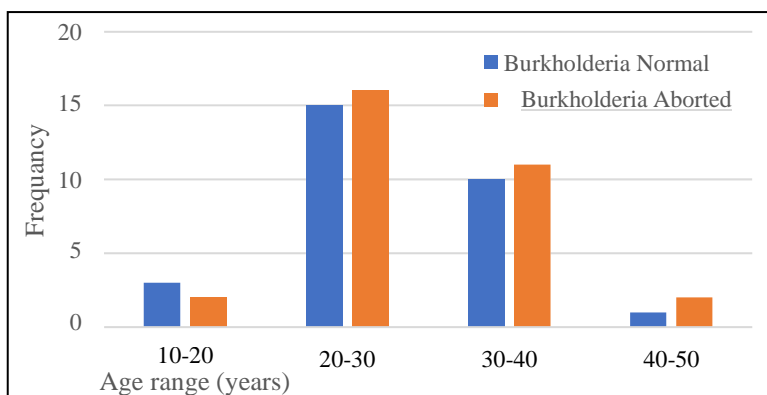
Mean(year)	28.5
Rural	28.21
Urban	28.83

The study revealed that highest rate of isolated bacteria from aborted women was *Burkholderia.cepacia* (31%), *E. coli* (22%) *klebsiella spp* (5%). *Pantoea* (10%) and the higher rates of isolated bacteria from pregnant women were 31% for *B.cepacia*, Table 2.

Table 2: Distribution of isolated pathogenic bacteria among study groups

Isolateed bacteria	Aborted women		Control women	
	No	%	No	%
B.cepacia	31	31%	29	29%
E.coli	22	22%	20	20%
Klebsiella ssp	5	5%	12	12%
Pantoea	10	10%	9	9%
No growth	32	32%	30	30
Total	100	100%	100	100%

Figure 1: show that the highest number of bacteria B.cepacia in abortion women for ages(20-30)



In this study, we have found some types of bacteria that cause infections in pregnant women that can to causing abortion, as well as the findings of Fouks et al Pregnancy loss through miscarriage or stillbirth (defined as intrauterine fetal death after 20 weeks).can also be caused by infection. Approximately 10–30% of all stillbirths have an infectious etiology (14). These changes may be the result of several factors as indicated by Amir et al. related to maternal immune responses, metabolism, and susceptibility to infections during pregnancy (15).

In many cases, the reasons for miscarriage remain unknown. however, aberrant chromosomal abnormalities were present in about 50% of early miscarriages.in the shape or quantity of chromosomes of the aborted fetus (16). The prevalence of vaginal infections was consistent with many studies conducted, where it was found that the prevalence of bacteria that cause female infection is higher in cities, which explains what we obtained from the percentage of aborted women in the city, which is slightly higher than in the city. villages, where the percentage of rural women who had abortions was 28.21%and the percentage of urban women who had

abortions was 28.83% Ahmadnia et al. concluded that women living in urban areas who had vaginal discharge had a higher risk of genital infection (17). We found many bacteria that cause abortions such as E.coli, pantoea, and Klebsilla. In our study, we focused on the Burkholderia bacteria, as it appeared in a high percentage in aborted women The percentage of this bacteria in aborted women was higher than in pregnancy women, and the Burkolderia bacteria was associated with vaginitis, as it was concluded by JOUR et al.in ten studies followed by urinary tract infections of Burkholderia (18).

phac gene Identification of B. cepacia.

The phac gene with constant expression in both control and test microarrays, was used as a control for all quantitative PCR work and amplified using previously described primers (19).

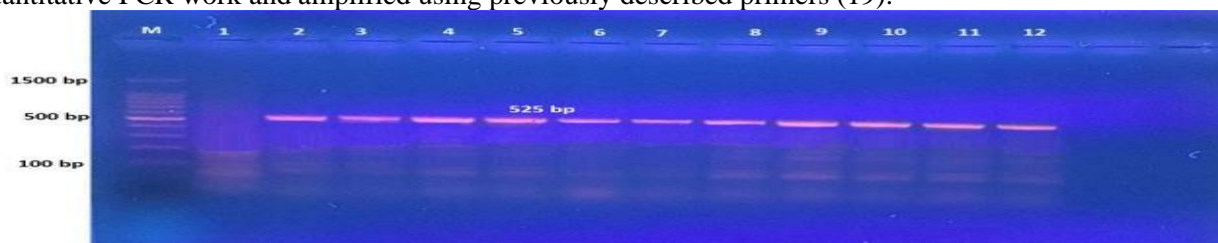


Figure 2: PCR results of *B. cepacia* isolates amplified with *phac* gene primers, having a product size of 525 bp. DNA molecular size marker Lane (L) (100-bp ladder).

RecA Gene Identification of B. cepacia

The *recA* gene was used to detect the bacterium *B. cepacia*. The present study found that the *recA* gene was found in samples tested using the VITEK 2 System, as shown in figure3.



Figure 3: PCR results of *B. cepacia* isolates amplified with *rec* gene primers, having a product size of 704 bp. DNA molecular size marker Lane (L) (100-bp ladder).

The *recA* gene has been widely utilized in bacterial systematics. It has proven to be particularly beneficial for identifying Bcc species. The *recA* gene with a phylogenetic analysis of sequence variation within the gene allows all nine current species within the Bcc to be distinguished.

However, the original *recA*-based PCR primers, *BCR1* and *BCR2*, are specific to members of the Bcc and do not amplify this gene in other *B. cepacia* species. While this can be a useful way of confirming an isolate's location within the complex, it limits the technique's application to classifying other *B. species* in different natural environments (20).

Gyr B Gene Identification of *B. cepacia*

The *gyrB* gene was used to detect the bacterium *B. cepacia*. The present study found that the *gyrB* gene was found in samples tested using the VITEK 2 System, as shown in figure 5.

The *gyrB* gene, encoding the subunit B of DNA gyrase (a type II topoisomerase), has been demonstrated to represent a useful molecular marker for phylogenetic and/or taxonomical analysis of genera *Pseudomonas* (21). *gyrB* gene, universally distributed among bacteria, as a new target gene to discriminate among the Bcc species. New PCR primers were designed to amplify a *gyrB* DNA fragment of about 1900 bp from 76 strains representative of all Bcc species (22).



Figure 4: PCR results of *B. cepacia* isolates amplified with *gyrB* gene primers, having a product size of 738 bp. DNA molecular size marker Lane (L) (100-bp ladder).

CONCLUSIONS

Bacterial vaginosis was common in aborted women and *Burkholderia* was the most isolated species followed by *E. coli*, *Pantoea* and *Klebsella* ssp. The identification of selected vaginal bacteria associated with an increased risk of miscarriage could support screening programs early in pregnancy and promote early therapies to reduce early pregnancy loss.

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