Identification of resistance genes of Burkholderia cepacia isolated from aborted women.

Mariam akeel[1] and Suaad abd alhadi[2]
Department of Biology, Faculty of Sciences, University of Kufa, Iraq
[1]mememo9966@gmail.com
[2]suaad.alhilo@uokufa.edu.iq

Abstract

Burkholderia cepacia complex (BCC) is a group of closely related bacteria with widespread environmental distribution. 200 samples were collected from women with abortion and pregnant 13–46 years of age, during the period from October 2022 to february2023. The results showed Burkholderia percentage of isolated bacteria from aborted women was *Burkholderia.cepacia* (31%), a non-probability study (purposive sample) of (100) women who had miscarriages was conducted as a study group they were selected from the emergency department and the maternity ward depending on the diagnosis of the doctor in charge, and (100) women with no history of abortion were selected as a control group from the department of Family and Child Care. The results of the study showed that the women of the study group were aborted women between the ages of (10-50) 47% of abortion cases were rural and 53% was urban, while the control was 28% from rural and 72% was urban. The genetic study of virulence factors, the identification of virulence genes, and conventional PCR testing method of work urine samples and vaginal swabs all subjects were within 13–46 years of age. 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* gene12 (100%) *gyrA* gene 6(50%),*phaC* 11(91.6), *gltB* gene 10(83.3), *atpD* gene 8(66.6).

Keywords: Bachoderia, Resistance genes, Abortedwomen

Introduction

Abortion and miscarriage are frequently used interchangeably. Abortion refers to the termination of a pregnancy, either artificially or naturally. A variety of terms are used to describe various stages of pregnancy loss. This category includes the phrases threatened, inevitable, full, and missed abortion. When the cervical canal is closed and a viable fetus is visible during a pelvic exam, this is a sign of threatened abortion (1).Abortion is one of the most common reproductive issues (2). Spontaneous abortion (SA) is one of the most common and serious problems during early pregnancy, accounting for 10-15% of all identified pregnancies (3).The definition of recurrent spontaneous abortion variesby country. For example, the American Society forReproductive Medicine defines recurrent spontaneousabortion as two or more failed pregnancies before20 weeks of gestation1and the European Society ofHuman Reproduction and Embryology defines recurrentspontaneous abortion as loss of two or more pregnan-cies.(4).Methods of identifying microorganism markers in the diagnosis and treatment of vaginal infections in pregnant women occupy a unique role in this regard (5). the interaction of a woman's microorganism with microorganisms involved in the development of the microbiome of the intestines, vagina, and other organs is largely responsible for the likelihood of conceiving and carrying a pregnancy (6).Infections caused by P. aeruginosa strains can be fatal due to a variety of mechanisms for adaptation, survival, and resistance to many classes of antibiotics, and they are becoming a global danger to public health (7) when the fetus is fully developed, women who contracted bacteria during pregnancy run the risk of miscarriage or giving delivery too soon (8), with bacterial genital tract infections accounting for

166

the majority of these occurrences . gardnerella vaginalis, group B streptococci, Staphylococcus aureus, and urea plasma mycoplasma hominis are aggressive microorganisms that can infect the reproductive system, particularly the vagina, and replace lactobacilli. vaginal pH may shift from 3,8 to 7 as a result (9).Burkholderia cepacia was once thought to be a single bacterial species but has expanded to the Burkholderia cepacia complex (Bcc), comprising 24 closely related opportunistic pathogenic species. These bacteria have a widespread environmental distribution, an extraordinary metabolic versatility, a complex genome with three chromosomes, and a high capacity for rapid mutation and adaptation. additionally, they present an inherent resistance to antibiotics and antiseptics(10)

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 women. the sample include vaginal swabs from miscarriage female Al-zahraa Hospital and Al- furat Hospital during al hasan al ascary clincal. the period from (octoer 2022 to February 2023). 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, mannitol salt agar,

Bacterial isolation
The obtained sample should then be incubated on MacConkey Agar, Blood Agar, and (MSA)Mannitol Salt agar for 24 hours at 37°C. Following isolation, the colonies were cleaned and cultivated. dietary agar. Using sheep blood agar, the isolates' pure colonies were selected (11). The bacterial isolates were identified using genomic and visual characteristics. Bergey's Manual of Systematic Biochemistry describes the characteristics of biochemistry. Bacteriology. Gram staining was used to assess the cellular makeup of the isolate bacteria, and the VITEK2 compact system was used to identify them(12).

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

Results and Discussion
Age Categories and Percentages of the Groups
 The ages of patients infected abortion from 13 to 46 years old on average 28.5 years, whereas the ages of controls from 18 to 50 years old on average 28.1 years, with abortion in the first trimester

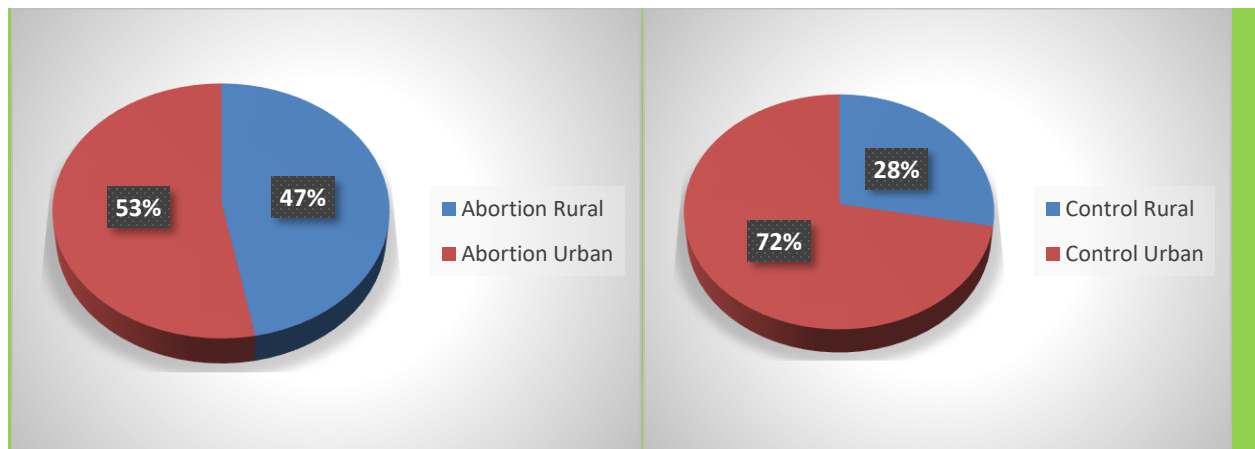| Variable	Study groups	No.	Range	Mean-SD	P-value
Age (years)	Patient	100	13-46	28.5±10.39	
	Control	100	18-50	28.1±26.07	0.143

Figure 1: of abortion cases 47% were rural and 53% was urban, while the control was 28% from rural and 72% was urban.
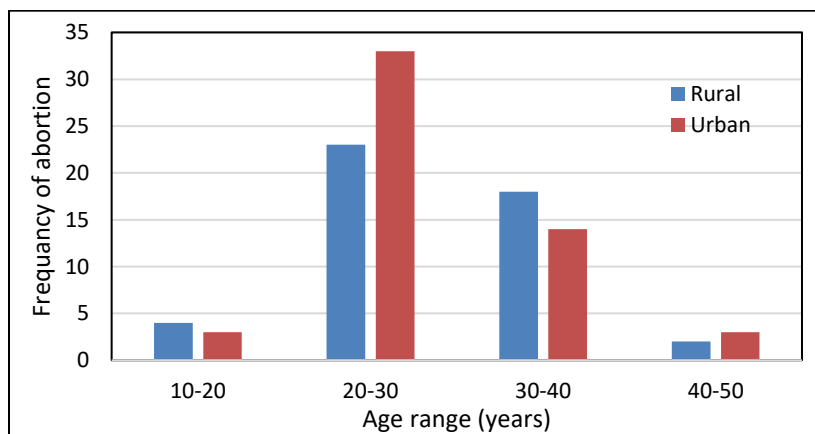

Figure2: attribute this percentage to the fact that rural women do not resort to the hospital in case of abortions, and because of their social status or lack of health awareness culture

Table 1: Distribution of isolation pathogenic bacteria among study groups

Isolated bacteria	Abortion women		Control women	
	No	%	No	%
B. cebacia	31	31	29	29
E. coli	22	22	20	20
Klebsiella ssp.	5	5	12	12
Enterobacter	10	10	9	9
No growth	32	32	30	30
Total	100	100	100	100

In this table, the pathogenic bacteria isolated from pregnant women and aborted women, where the percentage of E.coli bacteria was 22% higher in aborted women and 20% higher in pregnant women, as shown by research Ali et al.were found in his study This study was carried out in Azadi hospital in Kirkuk City, among pregnant women, from April 2002 to November 2002. A cross-sectional study included examination of 100 urine samples collected from pregnant women of age group 17-40 years attending Azadi hospital. The results clarified that urinary tract infections (UTIs) are among the most

168

common infections in pregnant women, since its prevalence was 29%. Symptomatic and asymptomatic UTIs scored 21% and 8% respectively. The most common pathogen causing UTIs was Escherichia coli and found Klebsiella bacteria was 12% higher in aborted women and 5% higher in pregnant women,and Enterobacter bacteria was 9% higher in aborted women and 10% higher in pregnant women(13).

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 describedprimers (14)
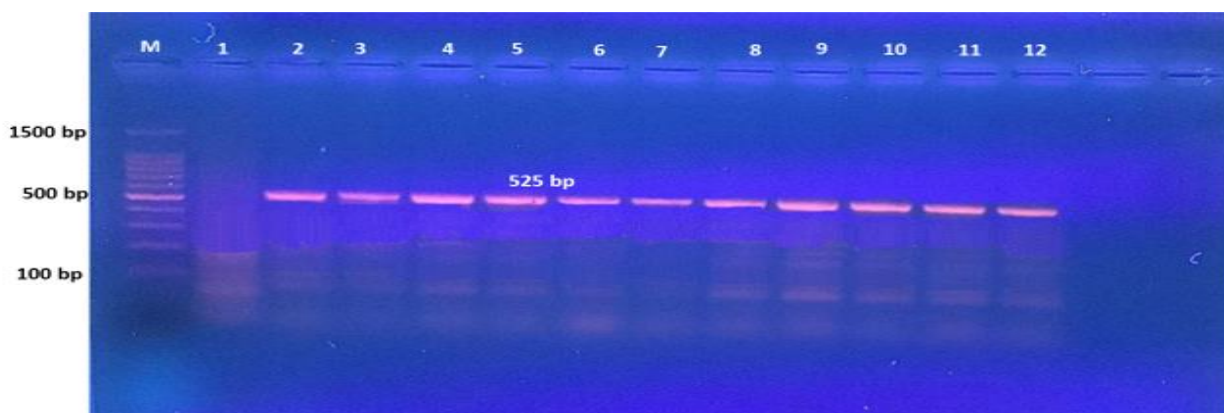

Figure3: PCR results of B.cepacia isolates amplified with phac gene primers, having a product size of 525 bp. DNA molecular size marker Lane (L) (100-bp ladder). degenerate primers for phaC gene based on multiple sequence alignment results and were designed for the second step of colony PCR to detect phaC,(15)

lepA Gene Identification of *B.cepacia*

The lepA gene was used to detect the bacterium B. cepacia. The present study found that the lepA gene was found in samples tested using the VITEK 2 System, as shown in figure4.
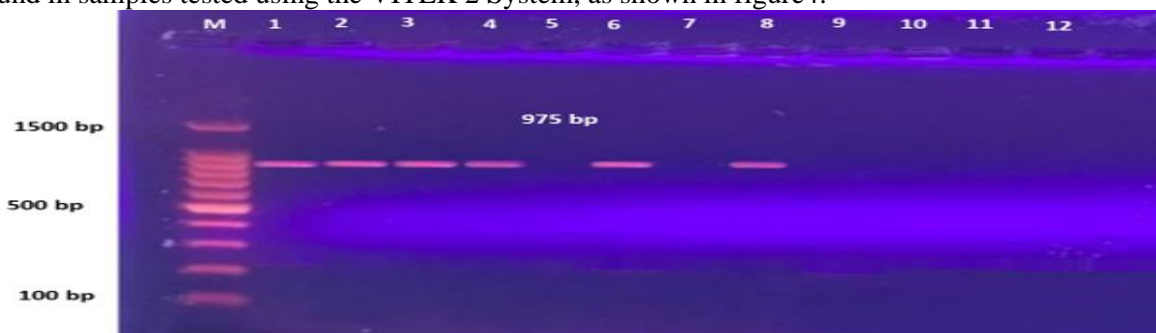

Figure4: PCR results of *B.cepacia* isolates amplified with lepA gene primers, having a product size of 975 bp. DNA molecular size marker Lane (L) (100-bp ladder).

TrpB Gene Identification of B. cepacia

The trpB gene was used to detect the bacterium *B.cepacia*. The present study found that the trpB gene was found in samples tested using the VITEK 2 System, as shown in figure5.
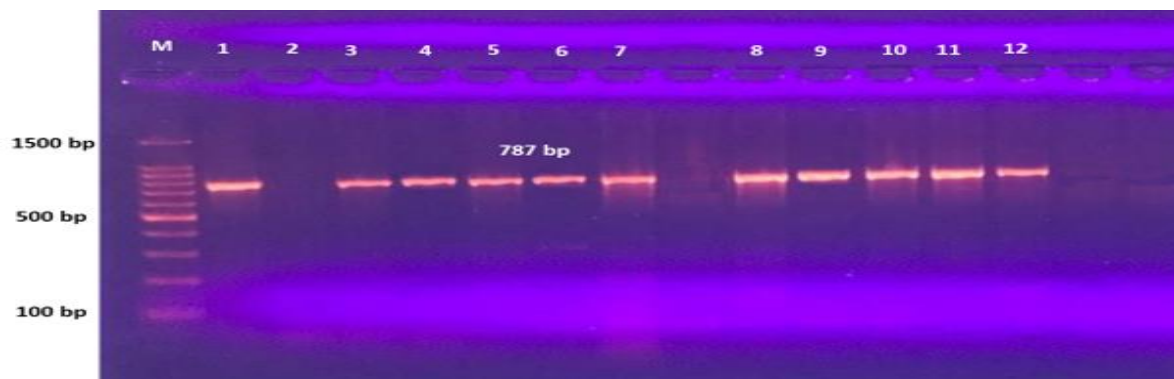
Figure5:PCR results of *B. cepacia* isolates amplified with *trpB* gene primers, having a product size of 787 bp. DNA molecular size marker Lane (L) (100-bp ladder).two variants of *trpB* (named trpB1 or trpB2) occur in different combinations.

The evolutionary history of these *trpB* genes is under debate.*trpB* genes may serve as a model system for studying the evolution of protein-protein interactions and operon formation(15).

Conclusions

Bacterial vaginosis was common in aborted women and Burkholderia was the most isolated to detect by pcr technology with genes of this bacteriaand 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.

References

1.Kanmaz, A. G., Inan, A. H., Beyan, E., & Budak, A. (2019). The effects of threatened abortions on pregnancy outcomes. *Ginekologia polska*, *90*(4), 195-200

2.Fathalla, M. F. (2020). Safe abortion: The public health rationale. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *63*, 2-12.

3.Rossen LM, Ahrens KA, Branum AM. Trends in Risk of Pregnancy Loss Among US Women, 1990-2011. Paediatr Perinat Epidemiol. 2018 Jan;32(1):19-29. doi: 10.1111/ppe.12417. Epub 2017 Oct 20. PMID: 29053188; PMCID: PMC5771868.

4.van Dijk LJEW, Nelen WLDM, D'Hooghe TM, DunselmanGAJ, Hermens RPMG, Bergh C, et al. The European Society ofHuman Reproduction and Embryology guideline for the diagnosisand treatment of endometriosis: an electronic guideline implement-ability appraisal. Implement Sci. 2011;6:7

5. Tait, E., Perry, J. D., Stanforth, S. P., & Dean, J. R. (2014). Use of volatile compounds as a diagnostic tool for the detection of pathogenic bacteria. *TrAC Trends in Analytical Chemistry*, *53*, 117-125.

6. Bezmenko A.A., Kislitsyna N.D. Intestinal dysbiosis - a risk factor or a direct cause of miscarriage? // Journal of Obstetrics and Women's Diseases. - 2018. - T. 67. - No. 2.- from 70–78.

7. Moradali, M. F., Ghods, S., & Rehm, B. H. (2017). Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. *Frontiers in cellular and infection microbiology*, *7*, 39

8.Summers, P. R. (1994). Microbiology relevant to recurrent miscarriage. *Clinical obstetrics and gynecology*, *37*(3), 722-729.

9. Casari, E., Ferrario, A., Morenghi, E., & Montanelli, A. (2010). Gardnerella, Trichomonas vaginalis, Candida, Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum in the genital discharge of symptomatic fertile and asymptomatic infertile women. *The new microbiologica*, *33*(1), 69.

170

10. Tavares, M., Kozak, M., Balola, A., & Sá-Correia, I. (2020). Burkholderia cepacia complex bacteria: a feared contamination risk in water-based pharmaceutical products. Clinical microbiology reviews, 33(3), e00139-19

11. Hemraj, V., Diksha, S., & Avneet, G. (2013). A review on commonly used biochemical test for bacteria. *Innovare J Life Sci*, *1*(1), 1-7.

12. Vos, M. (2020). The evolution of bacterial pathogens in the Anthropocene. Infection, Genetics and Evolution, 104611.

13. Ali, C., Anwar, S., Issa, S., & Khorsheed, S. (2007). Study of urinary tract infection among pregnant women in Kirkuk. Tikrit Medical Journal, 13(2), 39-43.

14. Baldwin, A., Mahenthiralingam, E., Thickett, K. M., Honeybourne, D., Maiden, M. C., Govan, J. R., ... & Dowson, C. G. (2005). Multilocus sequence typing scheme that provides both species and strain differentiation for the Burkholderia cepacia complex. *Journal of clinical microbiology*, *43*(9), 4665-4673.

15. Wong, M. Y., Tseng, Y. H., Huang, T. Y., Lin, B. S., Tung, C. W., Chu, C., & Huang, Y. K. (2020). Comparison of microbiological characteristics and genetic diversity between burkholderia cepacia complex isolates from vascular access and other clinical infections. Microorganisms, 9(1), 51.