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Molecular Study to Detect Some Virulence Factors in Vaginal Pathogenic Bacteria

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Abstract:
Background Bacterial vaginosis (BV) is a vaginal microbiome dysbiosis that is reported to be the most common gynecological condition in women of reproductive age (1). Methods: A total 120 swab samples were collected from patients with age 18 –50 years, during the period from 7th November, 2021 to 28th February, 2022 that admitted to Al-Zahra Teaching Hospital, Al Kut Hospital, , and private clinics in Wasit province. Bacteria were isolated and identified using biochemical test, then PCR was used in order to analysis he 16S rRNA gene in bacterial isolates and virulence genes using specific primers. Results: The results of bacterial cultures indicated the presence of four bacterial isolates included E.coli, K. pneumonia, E. faecalis and S. haemolyticus, PCR product analysis of the 16S rRNA gene in bacterial isolates showed that all bacteria 100% were positive for 16S rRNA gene. PCR analysis of virulence factor a total of E. coli, K. pneumoniae, E. faecalis, and S. haemolyticus isolates were confirmed by molecular technique using 16S rRNA. These concentrations and purity were identified by the Nanodrop technique. The concentration was between 50 and 360 ng/l, and the purity was 1.8 to 2.0. In the current study, thirteen virulence genes (fimD, rfbE, mrkD, ecpA, esp, gelE, mecA, icaD, icaA) were utilized and detected by polymerase chain reaction. Escherichia coli possessed 100% and fimD 42%, respectively. On the other hand, K. pneumoniae isolates expressed positive results for 90% of the ecpA and gene tests. However, possess revealed 90% for mrkD and 90% for genes. 83% of E. faecalis isolates tested positive for the esp gene and 83% tested positive for the gelE gene. Finally, S. haemolyticus isolates 100% and 100% for the mecA gene. Conclusion: There was a high prevalence of virulence genes among bacterial isolates included in the current study especially, in E.coli 100%, and E. faecalis, while S. haemolyticus icaD, icaA 0%.
Key words: Bacterial vaginosis, virulence factor sgenes PCR, BV.
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Bacterial vaginosis (BV) is a vaginal microbiome dysbiosis that is reported to be the most common gynecological condition in women of reproductive age (1). As well as BV has been recognized as one of the most perplexing mysteries in medicine and there is a large research gap around BV (2).

Understanding the disease of BV is critical for motivating research towards improved diagnosis and treatment of BV and its negative effects (3).There has been a significant rise in clinician and patient awareness of bacterial vaginosis as a common cause of vaginal discharge over the last decade. There has also been a lot of speculation about the involvement of BV in upper genital tract infections including pelvic inflammatory disease and premature labor and delivery (4).

In terms of aetiology and pathophysiology it is perplexing bacterial vaginosis is the most significant reason of vaginal discharge affecting 20-25% of the general population and up to 50% of women attending sexually transmitted diseases clinics (5) .

Regardless of the fact that studies have demonstrated that clinicians who make empirical diagnosis without the use of laboratory testing commonly misdiagnose the aetiology of a vaginal infection empirical diagnoses remain the standard in many practices. The most common signs of BV include odor and vaginal discharge. Pruritus is frequently not apparent (6).

In addition the bacterial vaginosis could be defined clinically or microbiologically. When three of the following four criteria are achieved the clinical or Amsel criteria allow for the diagnosis of BV (7).

(1) Vaginal pH greater than 4-5.

(2) Positive "whiff" test when vaginal fluid is combined with 10% KOH.

(3) Presence of clue cells contaminated squamous epithelial cells.

(4) Presence of a homogenous vaginal discharge microbiologically the BV syndrome is defined by a shift in vaginal microbes away from a lactobacillus-dominated flora to a vaginal environment dominated by anaerobes and facultative anaerobes such as Gardnerella, mycoplasmas, Prevotella/Porphyromonas, Mobiluncus and diminished numbers of lactobacilli, particularly those that produce hydrogen peroxide (8,9).

Material and Method

Study Design

A total 120 swab samples were collected by gynecologist from patients with age 18 –50 years, during the period from 7th November, 2021 to 28th February, 2022 that admitted to Al-Zahra Teaching Hospital, Al Kut Hospital, , and private clinics in Wasit province. Bacterial isolates were detected by culture characterization on the different media, biochemical investigation, analytical profile index 20 and Vitek2 automated system.

Statistical Analysis

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) software program version 26. All categorical variables were presented by frequency and percentages. Association between categorical variables was assessed by the Chi-Square test or Fisher's Exact Test (if > 20% of expected cell counts are less than 5 accordingly. Considering a *P*-value equal to or less than 0.05 as significant.

Primers

The following primers were employed in the current investigation, as shown in the Table (1) primers were generated using NCBI Gene bank sequence database design, online application, and these primers were produced by Alpha DNA, Canada, as reported in the Table1

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Table1: Sequences of primers used for detecting different bacterial isolates

NO.	Genes		Primer sequence (5´…3´)	Size (bp)	Reference
K. pneumoniae					
1.	16S rRNA	F	CCACACTGGAACTGAGACAC	298	NR_0367941
		R	TACCATCCGACTTGACAGAC		
2.	*mrkD*	F	GTTAAGTACACCTCCTACGAC	100	MK106182.1
		R	CGACATTCATATTTTTCCCGC		
3.	*ecpA*	F	GCGTCTTATCACCAACACC	127	CP091154.1
		R	ATCGCCAGTTTTTTCCACC		
Escherichia coli					
1.	16S rRNA	F	GCTAATACCGCATAACGTCG	297	LC682613.1
		R	ACTTTACTCCCTTCCTCCC		
2.	*fimD*	F	GTAATGGAATAACGCAGGGG	187	KM044268.1
		R	CAAAAGCACAGGCAACAAAC		
3.	*rfbE*	F	ACTACAGGTGAAGGTGGAATG	161	AF163336.1
		R	GCTAATCCTATAGCAGCGCAG		
Enterococcus faecalis					
1.	16Sr RNA	F	CAGCAAACGCATTAAGCAC	232	MH236316.1
		R	ACCCAACATCTCACGCAC		
2.	*esp*	F R	AAGAAGAGAGCGGAGACAC CTGAAATTGGAGCCCCATC	204	KF550185.1
3.	*gelE*	F R	ACCACGCATTAGCTTCAATC GCAGCAATCCATAGCAAGAAC	175	DQ845100.1
Staphylococcus haemolyticus					
1.	16S rRNA	F R	TGGAGAAATGTGAAGCGGG GCAGCAACAGTAACGGTAG	184	MF578766.1
2.	*mecA*	F R	GAGTAGCACTCGAATTAGGC AGTGAGGTGCGTTAATATTGC	240	MH553299.1
3.	*icaD*	F R	GCCCAGACAGAGGCAATATC ACAAACAAACTCATCCATCCG	228	FJ472951.2
4.	*icaA*	F R	CAAAATTAGGCGCAGTTACAG GCACCAACATCCAGCAAAG	297	FJ472951.2

Results

Molecular results

16S ribosomal RNA for *E.coli*

Diagnosis using PCR recorded *E. coli* as 10(100%), as shown in the Figure 1.

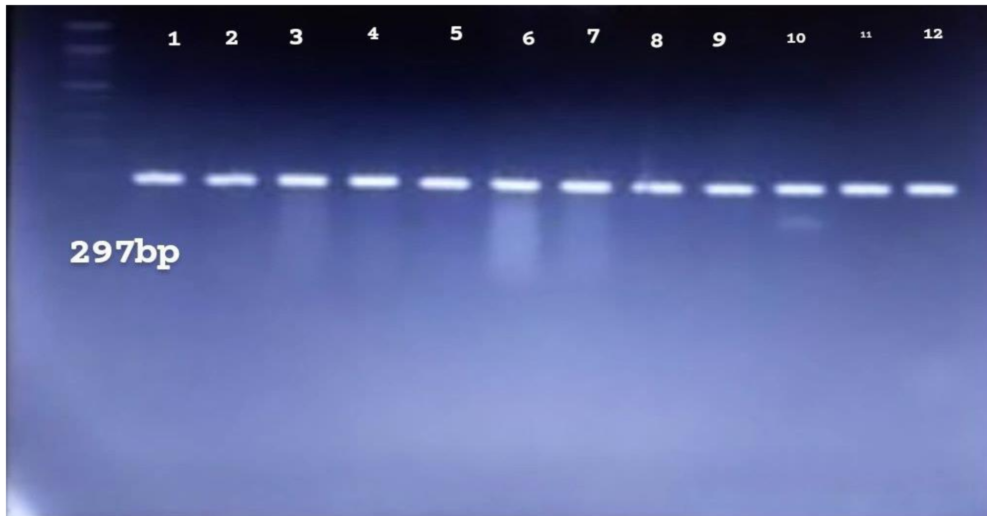

Figure1: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of *16S rRNA* gene in the *E .coli* isolates. Where, Lane of marker (M) ladder (200-10000bp), showed positive *16S rRNA* gene at 297bp PCR product size.

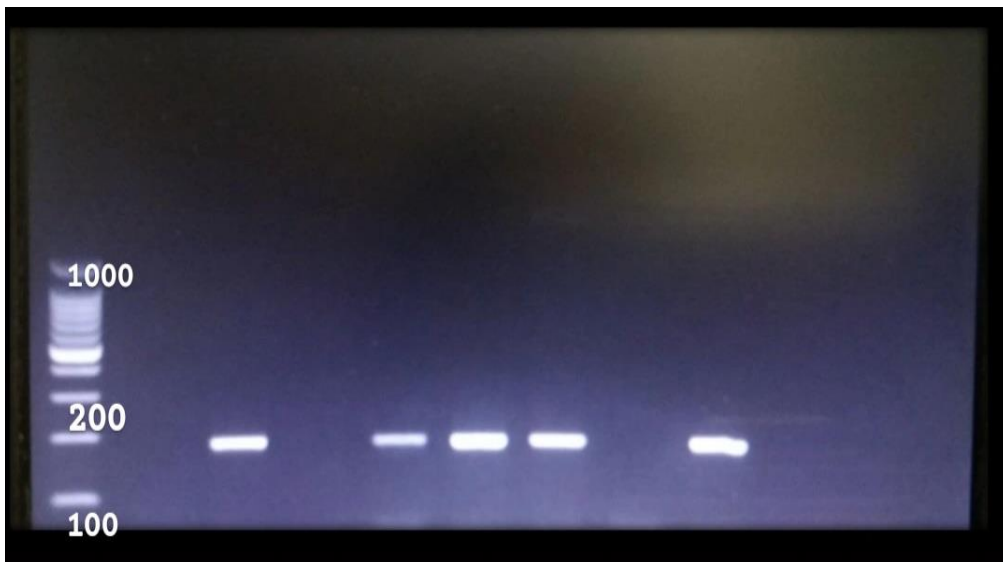

Figure 2: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of FimD gene in the *E .coli.* Where, Lane of marker (M) ladder (100-1000bp), at 187 bp PCR product size.

Figure 3: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of 16srRNA gene in the k. pneumonia Where, Lane of marker (M) ladder (200-10000bp), at 298 bp PCR product size.

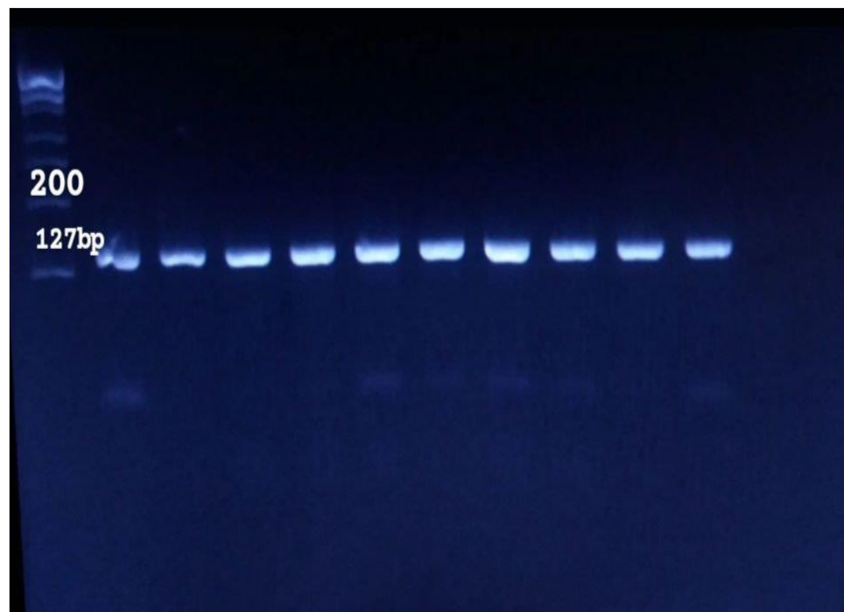

Figure 4: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of mrkD gene in the *k. pneumonia* Where, Lane of marker (M) ladder (100-1000bp), at 127 bp PCR product size.

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Figure 5: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of ecap gene in the k. pneumonia Where, Lane of marker (M) ladder (100-1000bp), at 100bp PCR product size.

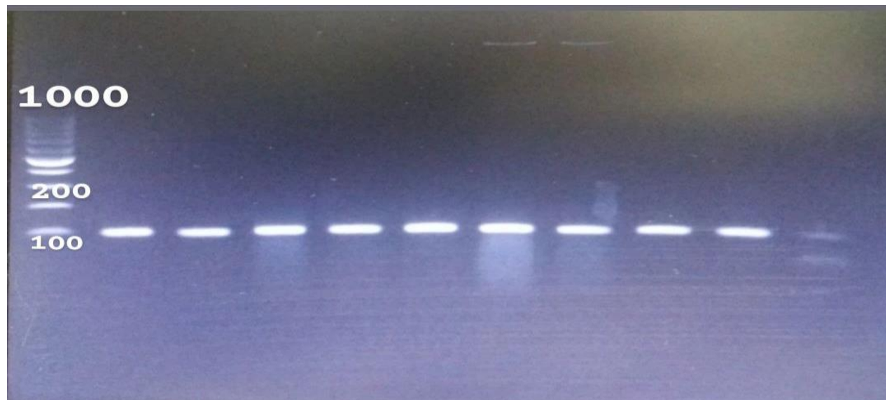

Figure 6: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of *16S rRNA* gene in the ***E***. faecalis isolates. Where, Lane of marker (M) ladder (100-2000bp), showed positive *16S rRNA* gene at 232bp PCR product size.

Figure 7: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of *16S rRNA* gene in the E. faecalis isolates. Where, Lane of marker (M) ladder (100-2000bp), showed positive gene gelE gene at 175bp PCR product size.

Figure 8: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of *16S rRNA* gene in the E. faecalis isolates. Where, Lane of marker (M) ladder (100-2000bp), showed positive gene *Esp* at 204bp PCR product size

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Figure 9: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of *16S rRNA* gene in the *S haemolyticus* isolates. Where, Lane of marker (M) ladder (100-2000bp),: showed positive *16S rRNA* gene at 197bp PCR product size.

Figure10: Agarose-gel-electrophoresis image that demonstrated the PCR product. Investigation of *16S rRNA* gene in the *S haemolyticus* isolates. Where, Lane of marker (M) ladder (200-10000bp), *MecA* gene 204bp

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Discussion

All of the bacterial isolates in this investigation tested negative for the rfbE gene, while 7 (58%) of the *E. coli* isolates tested positive for this *fimD* gene. These results were not in accordance with (10, 11) who indicated that *Escherichia coli* strains have emerged as important human enteric pathogens having the *FimD* gene in about 65% of the isolates, in which *Escherichia coli* is responsible for HAIs linked to genes, particularly the *fimD* and *rfbE* genes, rfbE (antigen gene), the most prevalent virulence genes in E. coli isolates were *(rfbE*) antigen gene (44 %). On the other hand the current study data were agreed to (12, 13) that *rfbE* gene encodes O-antigen in *E. coli* was highly prevalence in isolated bacteria (70%). Moreover (14) for simultaneous detection of *Escherichia coli* O157:H7, a multiplex polymerase chain reaction (PCR) test was designed in this work. The O157 antigen (*rfbE*) gene of *E. coli* O157:H7 was used to identify the six sets of specific primers for multiplex PCR. In the presence of bacterial DNA, the *16S rRNA* gene was selected as an internal control. All samples tested negative for the *rfbE*) gene, demonstrating that the new multiplex PCR assay is an efficient and informative addition for practical application. In current study, the results showed that *K. pneumoniae* showed that 9(90%) of the bacteria isolates were positive for both *E sap and mrkD genes*, our outcomes were similar to (15) showed that *E sap* and *mrkD* genes (90% and 89% respectively) helps bacteria connect with host cells, which increases their pathogenicity in the animal model. *K. pneumoniae* blood isolates were unable to attach to intestinal epithelial, lung epithelial, urinary bladder epithelial, or liver cells when their *esap* gene was deleted. These results shed light on how K. pneumoniae causes systemic infection by interacting pathogenically with the gastrointestinal system of the patient (16) When the expression of type *I fimbrial* genes (*mrkA* and *mrkD*) and the capsular polysaccharide synthesis (*cps*) gene *galF* were examined, the proportion of each was too low to draw any conclusions (20 %). While (17) study recorded that there was reduction of up to 50% in *mrkD* (type 3 fimbria adhesin subunit). This differences in these ratios for other studies may be due to more sampling. These conflicting results could be due to differences in the overall production of siderophores among the hvKp strains.

The results of the current investigation indicated that five (83%) of the E. cloacae isolates tested positive for the *Esp* and *geLE* genes. In (18) analysis of 212 isolates from 23 samples of rainwater tanks; testing for 6 virulence genes linked to *Enterococcus*-related illnesses. Twenty of the twenty-three rainwater tank samples (90%) included *E. cloacae*, seven (30%) contained *E.mundtii,* five (22%) contained *E. faecium*, two (9%) had *E.avium*, and one (45%) contained E. durans. Among the 6 virulence genes tested, *gelE* and *efaA* were most prevalent, 19 (83%) and 18 (78%) of 23 rainwater tank samples, respectively. The results in current study were in accordance with (19), who showed that *E. cloacae*, was screened for *gelE* and *esp* genes among *E. faecalis* , whose percentages for these genes were 93% and 92%, respectively. While our results were compatible with (20) indicated that resistance of *mecA*-positive isolates to wide-spectrum β-lactams was (100%). (21) Mentioned that lack of biofilm production by certain isolates despite their positivity for the *ica* genes like our findings, full phenotypic expression could be conditioned by genes expression *mecA* (100%).

While (22) All 106 *E. faecalis* isolates [26 human clinical isolates, 60 poultry clinical isolates (including two small-colony variations (SCVs)], and 20 poultry cloacal isolates were found to have the genes gelE and esp (23) *E. faecalis* accounted for 83% of the verified isolates and *E.*

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faecium for 17% of the isolates. *E. faecalis* had a biofilm forming capability of 91.5%, but *E. faecium* only managed 58.7%. The percentages of bacteria carrying the *gel E* and *esp* genes were 81.9% and 79.5% in *E. faecalis* and 76.5% and 58.8% in *E. faecium*, respectively. Additionally in (24) *Enterococcal* surface protein (*esp*) and Gelatinase virulence genes were checked for in a set of 13 antibiotic-resistant strains (*gelE*). There were six strains that tested positive for the *esp* gene (46.2%), and two strains that tested positive for the *gelE* gene (15.4%). These results not similar to results of present study. Different hospitals and locations may produce different results, which may be due to the large number of isolates, the methods used to control infection, or the drugs used to treat patients. All six bacterial isolates tested positive for the *mec* A gene, but all six tested negative for the *ica D* and *ica A* genes, indicating that *S. haemolyticus* in this investigation has the *mec A* gene but not the *ica D* or *ica A* genes. These data were not agreed with (25) as 100 isolates were included: 79.5% of isolates harbored the *icaD* gene, *icaA* and *icaB* genes were only found in CoNS (27.8% and 21.3% respectively). (20) Indicated that resistance of *mecA*-positive isolates to wide-spectrum β-lactams was (100%) (21)mentioned that lack of biofilm production by certain isolates despite their positivity for the *ica* genes like our findings, full phenotypic expression could be conditioned by genes expression *mecA* (100%).

Study showed that *S. haemolyticus* was assessed nasal colonization with CoNS. The Congo red agar technique was used to analyze biofilm development. Totaling 340 cotton swabs. Eighty-six percent of the isolates showed signs of MDR (multidrug resistance). The presence of the *mecA* (100%) and phenotypic resistance to methicillin, as well as the presence of the *icaA* (73%) and *icaD* (50%) genes, was found to be strongly correlated with biofilm development. (26) found that co-expression of some genes such as *icaA and icaD* are necessary for phenotypic expression of biofilm in many isolates,(27) who found that 68.2% of the clinical isolates were positive for the *ica* genes. A clear relationship was present between biofilm formation and detection f *icaA* and *icaD* genes among the tested strains in this study, which was statistically significant with the *icaA* gene (p=0.025). (28) Illuminated by the discovery of genes in *S. aureus* and *S. epidermidis* that induce a PIA-independent mode of biofilm formation. All devices were analyzed, and *S. haemolyticus*. were cultivated on chromogenic media and identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI), the results obtained showed that *S. aureus* was the dominant species (19 strains, 79.1%), followed by *S. warneri* (3 strains, 12.5%), and *S. haemolyticus* (2 strains, 8.3%). 37.5% of the isolates expressed the *mecA* gene. The tested strains were highly adhesive to polystyrene and glass and expressed implicated *icaA* (62.5%) and *icaD* (66.6%) genes. Many virulence genes were expressed, and biofilm formation was a common trait across the investigated strains. Characters from both DNA and phenotype were used in a phylogenetic analysis, revealing the population of S. aureus to be quite diverse. Additional studies are required to clarify the hazards posed by the detected Staphylococci strains to human health (29). Differences in these percentages may be due to other studies related to where the sample was taken and also to the nature and action of sterilizers and disinfectants used to combat hospital infections as well as personal hygiene and treatment methods.

Conclusions

There was a high prevalence of virulence genes among bacterial isolates included in the current study especially, in *E.coli* 100%, and *E. faecalis*, while *S. haemolyticus icaD, icaA* 0%.

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