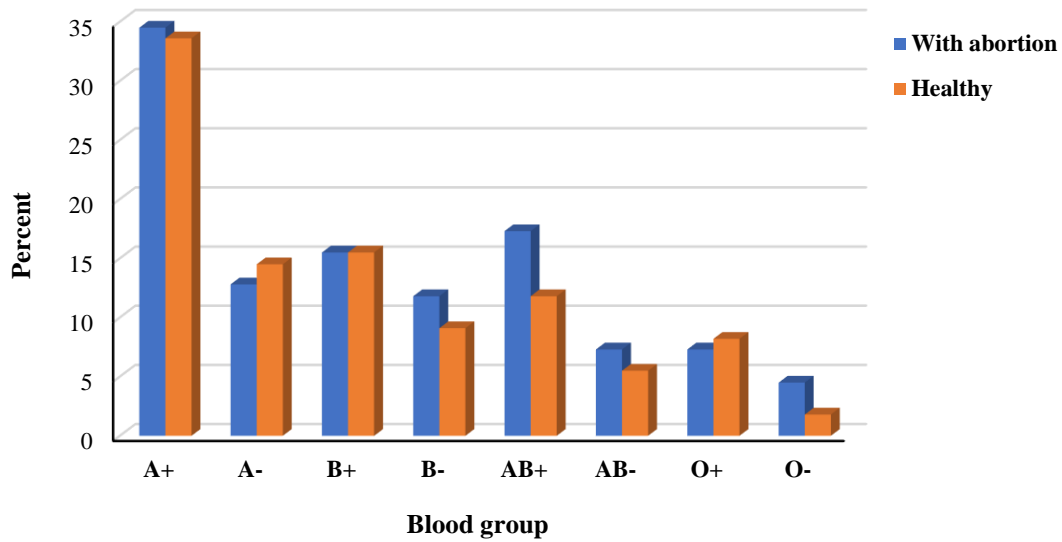


Appendix 1. The primers and reference strains used for molecular identification of the isolated bacteria by multiplex PCR, based on the 16S rRNA gene sequence

Primer	Sequence	Product length (bp)	Reference strain
FMHAB	5'- TCATCATGCCTCTTACGAGTG-3'	213	<i>Mycoplasma</i> <i>hominis</i> (Aj002268)
RMHCTAB	5'-TGTGACGGGCGGTGTGTAC-3'		
FLSAB	5'- TGACATCCTTTGACCACTCTG-3'	417	<i>Listeria</i> <i>monocytogenes</i> (NR_044823.1)
RMHCTAB	5'-TGTGACGGGCGGTGTGTAC-3'		
FSAAB	5'- GGAGCAGAAGTGACAGGTGG-3'	331	<i>Streptococcus</i> <i>agalactiae</i> (CP021773)
RSAAB	5'- GTGCTGATCCGCGATTACTAG-3'		

Appendix 2. Primer pairs used for the detection of *Streptococcus agalactiae* capsular antigen coding genes by multiplex PCR

Primer name	Sequence	Target gene	Sequence length (bp)	References
Ia-F	5'-GGTCAGACTGGATTAATGGTATGC-3'	cps1ah	521	(15)
Ia-R	5'-GTAGAAATAGCCTATATACGTTGAATGC-3'	cps1ah	1821	
Ib-F	5'-TAAACGAGAATGGAATATCACAAACC-3'	cps 1bj	770	(15)
Ib-R	5'-GAATTAACCTCAATCCCTAAACAATATCG-3'	cps 1bj		
II-F	5'-GCTTCAGTAAGTATTGTAAGACGATAG-3'	cps 2k	397	(16)
II-R	5'-TTCTCTAGGAAATCAAATAATTCTATAGGG-3'	cps 2k		
III-F	5'-TCCGTACTIONACAACAGACTCATCC-3'	cps 1a/2/3I	1826	(15)
III-R	5'-AGTAACCGTCCATACATTCTATAAGC-3'	cps 1a/2/3j		
IV-F	5'-GGTGGTAATCCTAAGAGTGAAGTGT-3'	cps 4N	578	(17)
IV-R	5'-CCTCCCAATTCGTCATACATAATGGT-3'	cps 4N		
V-F	5'-GAGGCCAATCAGTTGCACGTAA-3'	cps 5O	701	(15)
V-R	5'-AACCTICTCCTCACACTAATCCT-3'	cps 5O		
VI-F	5'-GGACTTGAGATGGCAGAAGGTGAA-3'	cps 6 I	487	(16)
VI-R	5'-CTGTCGACTATCCTGATGAATCTC-3'	cps 6 I		
VII-F	5'-CCTGGAGAGAACAATGTCCAGAT-3'	cps 7M	371	(18)
VII-R	5'-GCTGGTCGTGATTCTACACA-3'	cps 7M		
VIII-F	5'-AGGTCAACCACTATATAGCGA-3'	cps 8J	282	(17)
VIII-R	5'-TCTCAAATTCGCTGACTT-3'	cps 8J		

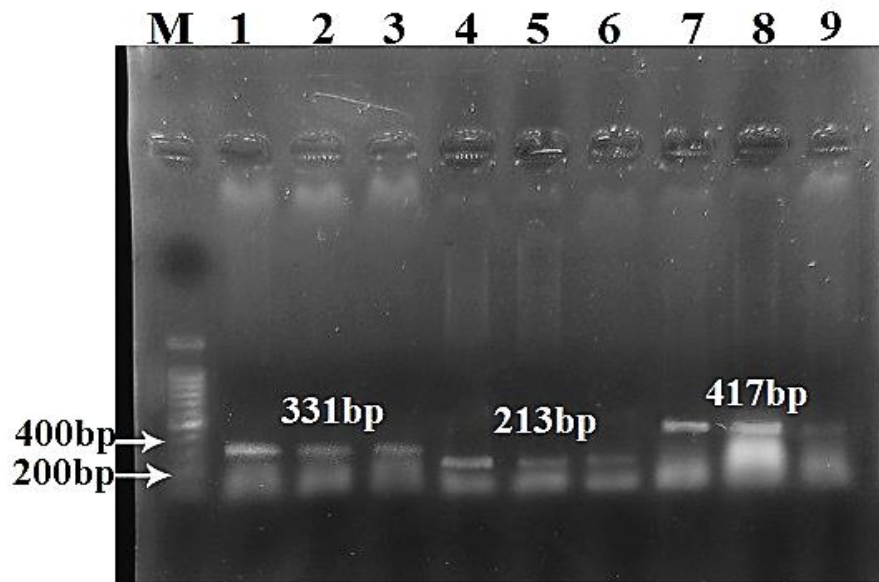


Appendix 3. Frequency distribution of blood groups in two groups of patients and healthy pregnant women

Appendix 4. The statistical analysis of some risk factors in two groups of patients and healthy women

Variable	Group	Healthy pregnant		With abortion		Statistics	P value	dds ratio (95% confidence)
		No.	Percent	No.	Percent			
Wife education level	Highschool under graduated	7	6.4	16	14.5	3.938	0.140	1
	Highschool graduated	52	47.3	47	42.7			
	Academic educated	51	46.4	47	42.7			
Husband education level	Highschool under graduated	11	10	19	17.3	12.443	0.02	1
	Highschool graduated	35	31.8	53	48.2			
	Academic educated	64	58.2	38	34.5			
Economic status	Low	23	20.9	21	19.1	0.502	0.778	1
	Average	68	61.8	66	60			

	High	19	17.3	23	20.9			1.326 (0.568-3.096)
Cesarean section history	No	76	61.9	71	64.5			1.228 (700-2.154)
	Yes	34	30.9	39	35.5	0.513	0.474	
Over weight	No	53	48.2	48	43.6	0.499	0.457	1.201 (0.706-2.042)
	Yes	57	51.8	62	56.4			
Stress	No	12	10.9	66	60	0.530	0.001	4.444 (2.674-11.080)
	Yes	98	89.1	44	40			
Vitamin D level	Deficiency	20	18.2	47	42.7	15.645	0.009	0.298 (0.161-0.551)
	Normal	90	81.8	63	57.3			



Appendix 5. The results from applying multiplex PCR reaction on three dilutions prepared from the extracted DNA of target bacteria in 1% agarose electrophoresis gel. The lanes 1-3 are the amplicons from DNA dilutions of 10 ng/ μ l, 5 ng/microliter, and 1 ng/microliter, respectively which was extracted from *Streptococcus agalactiae*, where a 331 bp band is seen. The lanes 4-6 are the amplicons from the same concentrations of the extracted DNA from *Mycoplasma hominis*, where a 213 bp band is seen. The lanes 7-9 are the amplicons from the same concentrations of the extracted DNA from of *Listeria monocytogenes*, a 417 bp band is seen. M: 100 bp DNA marker. The smear observed under the bands is related to possible amplification of the primer's dimers, which is common in multiplex PCR.