



Bacterial isolation of human follicular fluid and potential impact on *in vitro* fertilization outcomes

Samah Omar Noor^{1, 2}✉

¹Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

²Microbiology Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

✉ **Correspondence to:**

Samah Omar Noor, Department of Biological Sciences,
Faculty of Science, King Abdulaziz University, Jeddah,
Saudi Arabia,
E-mail: sonoor@kau.edu.sa

Article History

Received: 26 May 2020

Reviewed: 27/May/2020 to 07/July/2020

Accepted: 08 July 2020

E-publication: 12 July 2020

P-Publication: September - October 2020

Citation

Samah Omar Noor. Bacterial isolation of human follicular fluid and potential impact on *in vitro* fertilization outcomes. *Medical Science*, 2020, 24(105), 2782-2791

Publication License



This work is licensed under a Creative Commons Attribution 4.0 International License.

General Note



Article is recommended to print as color digital version in recycled paper.

ABSTRACT

Infertility is a global reproductive health issue impacting individuals and society and recognized as a cause for concern in Saudi society. It is medically defined as a failure to achieve conception after one year or more of regular unprotected sexual intercourse. Therefore, a lot of couples choose to have *in vitro* fertilization (IVF) procedure. Follicular fluid (FF) is one of the potential sources of microorganisms that may affect *in vitro* fertilization (IVF) outcomes. *Aim:* This study investigated the bacterial isolates within 50 FF samples of women undergoing IVF procedure. *Methodology:* All samples were identified according to morphological features, biochemical tests, and molecular techniques. *Results:* Nine bacterial species belong to five genera were detected within 38% of FF

samples and 62% did not show any bacterial growth. The bacterial species were identified as *Bacillus subtilis*, *Corynebacterium freneyi*, *C. amycolatum*, *C. aurimucosum*, *Enterococcus faecalis*, *Streptococcus anginosus*, *S. agalactiae*, *Staphylococcus aureus*, and *S. epidermidis*. The FF microorganisms were not associated with IVF outcomes. **Conclusion:** Follicular fluid is not sterile but have no significant adverse effects of FF microbes on the IVF outcomes. Furthermore, microbiological analysis of FF samples for women undergoing IVF procedure may give an opportunity to initiate antimicrobial treatment prior to the next conception.

Keywords: Follicular fluid, *In vitro* fertilization, Oocyte retrieval, Assisted reproductive technology, and Infertility

1. INTRODUCTION

Infertility is medically defined as a failure to achieve conception after one year or more of regular unprotected sexual intercourse (World health organization, 2016). It is a global reproductive health issue impacting individuals and society and recognized as a cause for concern in Saudi society (Kumar & Singh, 2015). It can be either primary infertility describes a couple who has never achieved conception for at least one year or secondary infertility, which is difficulty conceiving for a couple who have previously conceived (Anwar& Anwar, 2016).The prevalence of infertility estimated to affect as many as 48.5 million couples worldwide. This is especially prevalent in some regions including South Asia, North Africa, the Middle East, and Central Asia (Mascarenhas et al., 2012). In 2012, the prevalence of infertility was 18.93% in Alkhobar, Saudi Arabia, which was higher than in developed countries 3.5% to 16.7% within 12 months (Al-Turki, 2015). The common causes of female infertility include polycystic ovary syndrome, endometrioses, endocrine dysfunction, tubal factors, and pelvic inflammatory disease (Anwar & Anwar, 2016). Additionally, many lifestyle factors such as age, obesity, smoking, and others can impact overall health and contribute to infertility (Homan et al., 2007). Therefore, the couple goes to treatment using Assisted Reproductive Technology (ART) to get a child. This technology includes procedures such as *in vitro* fertilization (IVF) procedure (Aoun&Moawed, 2012). The routines of microbiological tests are not performed Prior to IVF cycles (Pelzer, 2011). One of the potential adverse impacts when the microbes transmitted from the contaminated embryo culture media into the female reproductive system that can lead to negative pregnancy outcomes. Many cases of this problem are due to a follicular fluid (FF) microorganisms (Pomeroy, 2010). It is a complex biological fluid that surrounds the developing oocyte in an ovarian follicle and plays a very important function for the development of the oocyte (Basuino & Silveira, 2016; Revelli et al.,2009). The FF can be colonized or contaminated by microorganisms that can effect embryo culture medium and may result in gametes DNA fragmentation, poor quality embryos, and preterm birth (Pomeroy, 2010; Borges & Vireque, 2019; Pelzer et al., 2013). Some microorganisms may be in low concentrations that influence the embryo culture medium but do not produce any apparent signs (Pomeroy, 2010). Traditionally, the embryo culture media contains antimicrobial agents such as gentamycin to prevent the growth of pathogenic microorganisms while some types of bacteria can be a resistance to these particular antibiotics (Borges & Vireque, 2019; Pelzer et al., 2012). Furthermore, antimicrobial agents in the culture medium provide a little inhibition to the potentially large number of bacteria (Moore, D., et al., 2000). These points of concern may lead to a decrease in IVF outcomes.

Previous studies reported human FF microorganisms and their association with negative IVF outcomes (Pelzer, 2011; Pelzer et al., 2013; Hamad et al., 2018; Kimet et al., 2018). According to our knowledge in Saudi Arabia, similar studies have not been performed yet, and due to the high number of infertility people, this type of study is important and will contribute to providing important information that will help doctors to improve IVF procedure.

The aims of this study were to identify the bacteria in FF of women undergoing IVF procedure at the time of oocyte retrieval and the correlation of these microbes with IVF outcomes and to study the effects of some antimicrobials agents on bacterial isolates.

We hypothesized that FF bacteria may have an effect on the success rate of the IVF procedure.

2. METHODS

Specimen collection

From October 2018 to April 2019, we conducted a cross-sectional study of 50 female participants who undergone IVF procedure at the time of oocyte retrieval. Ethics approval was obtained from the Biomedical Ethics Unit at King Abdulaziz University Hospital, Jeddah, Saudi Arabia. All participants signed informed consent forms and gave permission to use their medical records.

The inclusion criteria are fertile women who have an infertile male partner (male factor), and infertility women with different causes including (ovulatory dysfunction, endometriosis, tubal factor, and unexplained infertility). The exclusion criteria are women using antibiotics for one month prior to sampling.

Microbial culture

Undiluted 50 FF samples were inoculated directly by 1 μ L loops using a streak technique in triplicate on top of the deferential and selective media (blood agar (BA), MacConkey agar (MAC), chocolate agar (CHOC), de Man Rogosa Sharpe agar (MRS); Himedia, Mumbai, India). All plates were incubated aerobically in 5% carbon dioxide incubator (Sanyo Com., Osaka, Japan) or anaerobically in anaerobic jars (OxoidAnaerobic 3.5L Jar, Massachusetts, USA) at 37°C for 48 hours. All procedures of microbial isolates were performed under sterilized conditions inside the Biosafety cabinet (Daihanlabtech, Kyungki-Do, South Korea).

Morphological structures analysis

All bacterial isolates were phenotypically identified from pure cultures grown on Nutrient agar medium (NA) (HiMedia, Mumbai, India), Colony features including growth rate, colony color, texture, size, and shape were recognized as important diagnostic criteria for identification. The microscopic appearance was observed in wet mounts prepared on microscopic slides and stained with Gram stain using the standard protocol (Carter & Cole, 2012).

Microbial analysis

The total number of colonies was manually counted and the results were calculated as the mean of colony forming units per milliliter (CFU/ml) as described by Pelzer (2011).

Biochemical analysis

Different biochemical analysis tests conducted to differentiate between the bacterial colonies including Catalase, Coagulase, Mastastrep, and Starch hydrolysis tests (Hemraj et al., 2013). Catalase-positive cocci were identified by coagulase test (Oxoid) to differentiate between *Staphylococcus aureus* and other types of Staphylococci. Catalase-positive bacilli were confirmed by Starch hydrolysis test, and catalase negative-cocci were identified by Mastastrep latex agglutination test (Mast group Ltd., UK) to differentiate between *Streptococcus* species.

For long term storage, all the isolates were preserved at -80°C in Nutrient broth (NB) (Micromaster, Maharashtra, India) containing 20% (1:1) sterile glycerol (Sigma-Aldrich, Missouri, USA), (Bergey et al. 2001).

Molecular identification of isolated bacteria

DNA extraction and PCR amplification

The bacterial isolates were individually inoculated in 5 ml of NB medium and incubated in shaking incubator (SI-100, Human Lab, Gyeonggi-do, South Korea) at 37°C to a density corresponding to 2.0 McFarland using spectrophotometer instrument (Genesys 10S UV-Vis, USA). The bacterial genomic DNA was extracted using QiAMP mini DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Polymerase chain reaction (PCR) assay was performed to amplify 16S ribosomal RNA (rRNA) using universal primer; the forward primer (27-f) (5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse primer (511-r) (5'-GCGGCTGCTGGCACRAGT-3') in thermal cycler (Veriti Thermal Cycler, Applied Biosystems, USA) (Alnefaie et al., 2019). The reaction mixture (50 μ l) included: 1 μ l of templet DNA, 2 μ l of each primer (Macrogen, Seoul, South Korea). 25 μ l of green PCR mix (Promeaga, Go Taq® Green Master Mix, USA) and 20 μ l of nuclease-free water. The PCR program was as follows: the initial cycle of 94 °C for 5 min, followed by 32 cycles of 45 s at 94°C, 60°C for 45 s, and 72°C for 90 s, with a final extension at 72 °C for 10 min. The negative control was prepared with the reaction mixtures in the absence of DNA extract (Alnefaie et al., 2019). The DNA concentration and purity were measured at 260 and 280 nm using Nanodrop spectrophotometer (Nanodrop 2000, Thermo scientific, Massachusetts, US).

DNA Visualization

The PCR products were loaded onto 1.5% agarose gel prepared in 1X Tris Acetate-EDTA (TAE) buffer (Thermo Scientific, Massachusetts, US). Agarose was dissolved with ethidium bromide added to a final concentration of 1 μ g/ml. Amplicons were run for 45 min at 130 volts using electrophoretic gel (Horizontal gel electrophoresis, Cleaver Scientific, UK) and visualized under UV light (versadoc imaging system, Bio-Rad, USA) to evaluate the DNA quality. The DNA marker (100 bp, Invitrogen, USA) was used to identify and quantify PCR products. Then the samples were sent to Macrogen Company, South Korea for purification and sequencing.

DNA Sequencing

The sequence results were assembled using big dye terminator cycle sequencing kit (Applied Biosystems, U.K). Sequence identities were characterized using the Basic Local Alignment search tool (BLAST general GenBank databases from the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>). The sequence alignment of the neighbor analysis structures was done by MEGA-X program. The sequencing data were submitted to GenBank and the accession number obtained and recorded for each bacterial isolates.

Antimicrobial susceptibility test

The susceptibility to seven antimicrobial agents was determined using the Kirby-Bauer agar disk diffusion method. The following antimicrobial agents were obtained from (Oxoid, Hampshire, U.K.), Amoxicillin-clavulanic acid (AMC) 20/10 µg, Cefepime (FEP) 30 µg, Erythromycin (E) 15 µg, Metronidazole (MET) 5 µg, Doxycycline (DOX) 30µg, Gentamycin (GN) 30 µg, and Clindamycin (DA) 10 µg. A pure single colony of each isolate was picked and transferred into 2 ml of sterile saline to prepare the bacterial suspension. The suspension was adjusted to match the 0.5 McFarland turbidity standards using spectrophotometer instrument (Genesys 10S UV-Vis, USA). Then, aliquots of 100 µl from each suspension were spread-plated on Mueller hinton agar (MHA) (Himedia, Mumbai, India). After that, the antibiotic disks were applied on the top of plates using the Oxoid Disk Dispenser and the plates were incubated at 37 °C for 18-24 hours. After incubation, the antibiotic inhibition zone diameters (IZD) were measured in millimeters (mm) (Hudzicki, 2009). Finally, the results obtained were used to classify isolates as being susceptible, intermediate or resistant to a particular antimicrobial agent according to Clinical and Laboratory Standards Institute documents (Clinical Lab Standards Institute, 2016).

Statistical Analyses

Data collected were analyzed using SPSS 25.0 to determine the significant difference among the data (Armonk, NY: IBM Corp). The data were tested using Shapiro–Wilk tests, Chi-square test (χ^2), Kolmogorov–Smirnov test, T-test, and ANOVA test. A p-value of <0.05 was considered statistically significant.

3. RESULTS

Patient demographics

Clinical specimens were collected from 50 women for microbiological analyses. The mean (\pm SD) age of all women was (35.8 \pm 5.47) years. Overall cases, the percentage of women with different causes of infertility was 68% and for fertile women (male factor) was 32%. The ovulatory dysfunction consist most of infertility causes 44%, followed by male factor 32%, unexplained infertility, tubal factor, and endometriosis consist 12, 10 and 2% respectively. The positive IVF outcomes were 28% while 72% were the negative IVF outcomes. Additionally, the IVF outcomes were not correlated with the aetiology of infertility ($P>0.05$) and ovulatory dysfunction were the most common causes of infertility with negative IVF outcomes 38% as shown in Table [1].

Table 1: The relation between the etiology of infertility and IVF outcomes

Aetiology of infertility	IVF outcomes		P-value
	Positive 14 (28%)	Negative 36 (72%)	
Fertile women (Male factor)	8 (16%)	8 (16%)	0.125ns
Ovulatory dysfunction	3 (6%)	19 (38%)	
Tubal factor	2 (4%)	3 (6%)	
Endometrioses	0 (0%)	1 (2%)	
Unexplained infertility	1 (2%)	5 (10%)	

ns: non-significant

Isolation and identification of microbial isolates

Microorganisms were detected within 19 (38%) FF samples and 31 (62%) did not show any bacterial growth. The microbial analyses revealed that there were (1-2 species) isolated from each FF sample. One species in 14 (28%) FF samples, and two species in 5 (10%) FF samples.

The rates of bacterial species isolated from FF in women with ovulatory disorder, unexplained fertility, male factor, and tubal factor were 13/22 (59%), 3/6 (50%), 7/16 (44%) and 0/5 (0%) respectively with no statistically significant difference ($P>0.05$). The presence of microbes in the FF and endometriosis were found to be associated ($P=0.002$).

In the microscopic specimens, 67 bacterial isolates were Gram-positive reaction and the morphological features were identified up to the genus level according to the criteria of Bergey's Manual of determinate Bacteriology (Vos et al., 2011).

The effect of microbial load was also considered in this study. The quantitative method assessed the prevalence of microorganisms within the FF samples. The bacterial species were isolated at concentrations ranging from 10^3 CFU/ml to $>10^5$ CFU/ml in FF samples. There appeared to be no correlations between the microbial load in FF and the aetiology of infertility. However, the FF microorganisms were not associated with IVF outcomes ($P>0.05$) as shown in Table [2].

All bacterial isolates were identified using the biochemical tests as seven isolates of *S. agalactiae*, two isolates of *S. aureus*, one isolate of other types of *Staphylococci*, three isolates of group F *Streptococci*, one isolate of *Enterococci*, three isolates of *Bacillus* spp. and seven isolates of *Coryne bacterium* spp.

Twenty four bacterial isolates from the FF were identified and confirmed using molecular analytical techniques. All bacterial isolates belongs to five genera and nine species which were identified as *Bacillus subtilis*, *Corynebacterium freneyi*, *Corynebacterium amycolatum*, *Corynebacterium aurimucosum*, *Enterococcus faecalis*, *Streptococcus anginosus*, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. *S. agalactiae* was the most prevalent species 29%, followed by *C. aurimucosum* 17% as shown in Figure [1].

Table 2: The relation between follicular fluid microorganisms and IVF outcomes

Bacterial species	IVF outcomes		P- value
	Pregnant	Non-pregnant	
<i>B. subtilis</i>	1 (2%)	2 (4%)	0.832
<i>C. freneyi</i>	0 (0%)	2 (0%)	0.368
<i>C. amycolatum</i>	1 (2%)	0 (0%)	0.529
<i>C. aurimucosm</i>	0 (0%)	4 (8%)	0.193
<i>E. faecalis</i>	0 (0%)	1 (0%)	0.529
<i>S. anginosus</i>	2 (4%)	1 (2%)	0.124
<i>S. agalactiae</i>	1 (12%)	6 (0%)	0.348
<i>S. aerues</i>	0 (0%)	2 (4%)	0.368
<i>S. epidermidis</i>	0 (0%)	1 (0%)	0.529
Total	5/14 (36%)	19/36 (53%)	0.381

Table 3: List of bacterial strains GenBank accession number isolated from FF and % identity with closely related strains found in the NCBI website

Accession number	Closely related strains accessed from GenBank	Similarity (%)
MT002916	<i>B. subtilis</i> KY613181.1	99
MT002842	<i>C. freneyi</i> EF462412.1	100
MT002872	<i>C. amycolatum</i> MN175937.1	99
MT002926	<i>C. aurimucosum</i> MN175932.1	99
MT002917	<i>E. faecalis</i> MN894281.1	100
MT002833	<i>S. anginosus</i> MH628245.1	99
MT002828	<i>S. agalactiae</i> MN267810.1	99
MT002876	<i>S. aureus</i> LT689037.1	100
MT002925	<i>S. epidermidis</i> MK611794.1	100

The PCR amplification of DNA extracted from the bacterial strains was done with universal primers pair. The intense sharp bands on agarose gel appeared the PCR product with a molecular size of 16S rRNA region nearly 500 bp for all bacterial strains as shown in Figure [2]. This was followed by PCR genetic sequences of all strains that were aligned with the available sequences of closely related strains accessed from the GenBank. Molecular identification of strains to species level based on 99–100% similarity with sequences of the known species already published in NCBI databases. Sequences were also submitted to GenBank and given accession numbers as shown in Table [3].

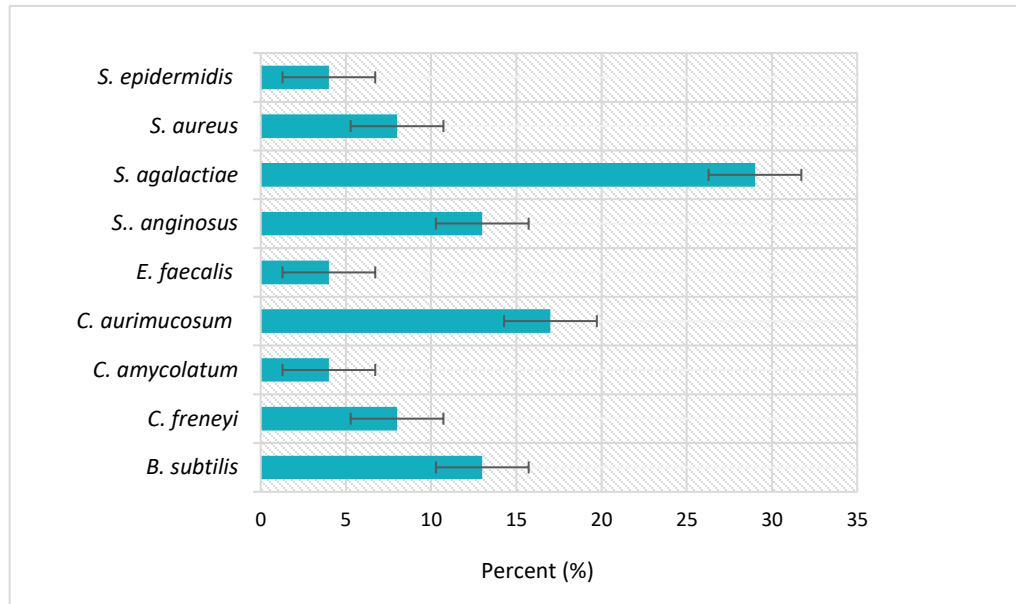


Fig. 1: The percentage of bacterial species detected within the FF samples. Graph showing that the predominant species was *S. agalactiae* (29%), followed by *C. aurimucosum* (17%). The other microbial species were *S. anginosus*, and *B. subtilis*, (13%), *C. freneyi*, and *S. aureus* (8%), *S. epidermidis*, *E. faecalis*, *C. amycolatum* (4%).

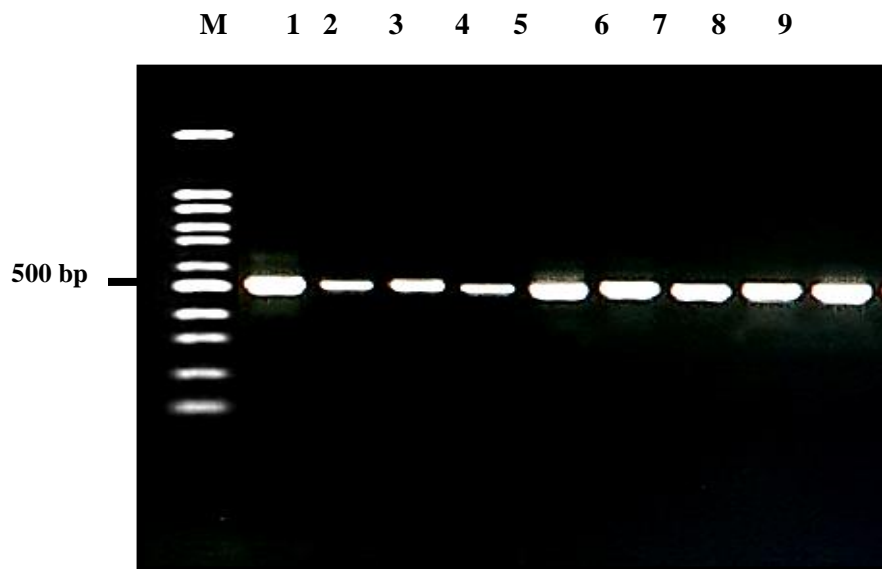


Fig. 2: Gel electrophoresis of PCR product of nine bacterial species; all data shown were obtained with 16S rRNA primer. The range of DNA isolates (1 - 9) molecular size of bands was 500 bp compared to M (100 bp molecular size marker).

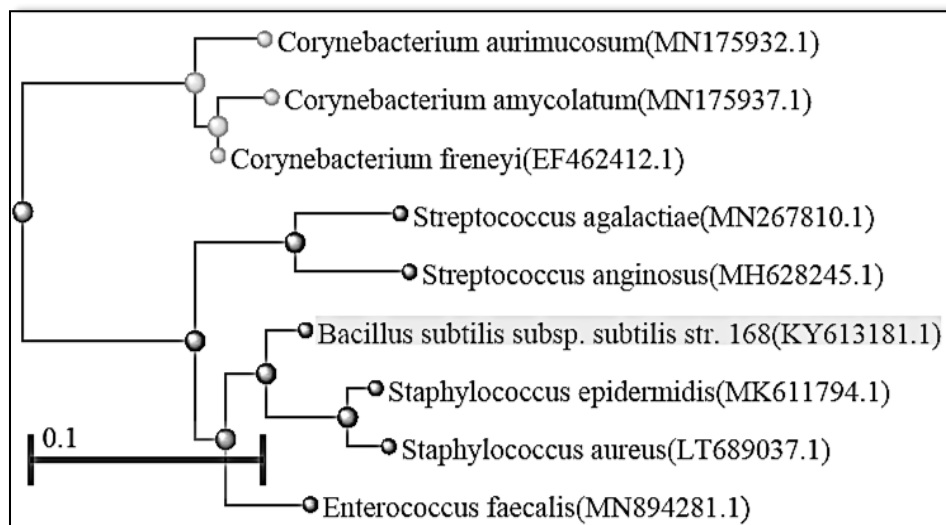


Fig. 3: Dendrogram showing phylogenetic analysis based on the 16S rRNA region and NCBI GenBank database for nine bacterial species.

The dendrogram showing phylogenetic analysis indicated the taxonomic relationship based on 16S rRNA region of the nine bacterial species aligned with closely related sequences accessed from the GenBank are illustrated in Figure [3]. The following bacterial isolates *S. anginosus*, *S. agalactiae*, *E. faecalis* belong to the same order which is Lactobacillales. While the *S. epidermidis*, *S. aureus*, and *B. subtilis*, followed the Bacillales order. In regard to *C. freneyi*, *C. amycolatum*, and *C. aurimucosum* the order is Corynebacteriales. All data obtained with 16S rRNA sequences of the bacterial isolates could be grouped into two phylum Firmicutes and Actinobacteria.

According to Clinical and Laboratory Standards Institute (CLSI) (Clinical Lab Standards Institute, 2016) documents, the bacterial isolates indicate different antimicrobial susceptibility to six antimicrobial agents including AMC, FEP, E, DOX, CN, and DA. Most of the bacterial isolates showed high susceptibility 67% to AMC and FEP, moderate susceptibility 21% to E and 100% resistant to MET, then 58% to CN as shown in Table [4].

Table 4: The percentage of antimicrobial susceptibility for the bacterial species isolated from FF samples.

Antibiotic agents	Disc concentration (µg/disc)	Total number of bacterial isolates (%)		
		S	I	R
AMC	20/10 µg	16 (67%)	0 (0%)	8 (33%)
FEP	30 µg	16 (67%)	0 (0%)	8 (33%)
E	15 µg	15 (63%)	5 (21%)	4 (17%)
MET	5 µg	0 (0%)	0 (0%)	24 (100%)
DOX	30 µg	15 (63%)	3 (13%)	6 (25%)
CN	30 µg	10 (42%)	0 (0%)	14 (58%)

(S) Susceptible; (I) Intermediate; (R) Resistant. Amoxicillin-clavulanic acid (AMC); Cefepime (FEP); Erythromycin (E); Metronidazole (MET); Doxycycline (DOX); Gentamicin (CN); and Clindamycin (DA).

4. DISCUSSION

This study investigated the presence of bacterial species within fifty FF specimens collected from women undergoing IVF procedure with different causes of infertility and the effect of these microbes on the IVF outcomes.

In the present study, five genera and nine bacterial species were identified from 19FF specimens. The bacterial isolates were present in 38% of FF while there were 62% of FF did not show any bacterial growth. The ovulatory dysfunction consist most of infertility causes 44%, followed by male factor 32%, unexplained infertility, tubal factor, and endometriosis consist 12, 10 and 2% respectively. We concluded that the presence of bacterial species (*S. anginosus*, *S. agalactiae*, *B. subtilis*, *C. freneyi*, *C. amycolatum*, *C. aurimucosum*, *S. aureus*, *S. epidermidis*, and *E. faecalis*) were not correlated with adverse IVF outcomes. In agreement with our findings, previous studies isolated different types of microbes in FF samples including (*Lactobacillus spp.*, *staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, and *E. faecalis*) with no significant adverse impacts on IVF outcomes (Hamadet al., 2018; kim, 2018). Other studies were incompatible with our findings, they demonstrated that the presence of FF microorganisms was associated with adverse IVF outcomes (Plezer, et al., 2011; Plezer, 2013; Ibadin & Osemwenkha, 2014). Additionally, another study reported that the FF microorganisms may result in a poor quality of oocytes that leading to reduced IVF outcomes (Pelzer, 2013).

The FF may be contaminated by vaginal microorganisms when the embryologist collects the sample by a needle passed through the vagina into the ovary (Aleshkin, 2006; Matytsina, 2010).It is may also be colonized by a hematogenous invasion of microorganisms spread via the oral mucosa and respiratory tract (Borges & Vireque, 2019). In the present study, *Staphylococcus spp.*, *Enterococcus spp.*, *Corynebacterium spp.*, and *Streptococcus spp.* isolated from FF samples appear to be opportunistic pathogens in the vagina according to the work published by others (Aleshkin, 2006; Matytsina, 2010). *S. agalactiae* is a main infectious reason for neonatal disease and death and it can be an association with the complications of pregnancy (Donatiet al., 2010; Stoll et al., 2011). We have also shown that FF microbes were not correlated with endometriosis. This is compatible with the findings of others who have demonstrated that there is a correlation between FF colonization by microorganisms and endometriosis (Plezer et al., 2013).

The bacterial isolated from FF samples recovered in this study at concentrations ranging from 10^3 CFU/ml to $>10^5$ CFU/ml and there was no correlations between the microbial load and the etiology of infertility. Another study showed that most microbial species were isolated at concentrations ranging from 10^3 CFU/mL to $>10^6$ CFU/mL in both colonized and contaminated FF and the microbial load was not associated with the aetiology of infertility or the IVF outcomes (Plezer et al., 2011).

All bacterial isolates were identified using the biochemical tests. To ensure suitable differentiation and confirmation of the isolates to the species level, we used the advanced molecular technique based on amplifying the 16S rRNA segment using PCR products and sequencing analysis method. Several researchers reported bacterial species based on this technique to confirm species identification (Alnefaie, 2019). The molecular identification for nine bacterial strains revealed that the identification of microbial isolates using the morphological features, the Gram stain, and the biochemical tests were correct, thus yielding the correct identification for all 24 bacterial isolates tested. For instance, consistent with our data which reported a molecular size of 16S rRNA region nearly 500 bp for all bacterial isolates were mentioned by (Chen et al., 2014)

Traditionally, the embryo culture media for IVF procedure contains antibiotics such as penicillin, streptomycin, or gentamycin as an attempt to prevent the growth of pathogenic microorganisms while some types of bacteria can be a resistance to these particular antibiotics (Borges & Vireque, 2019; Kumar & Singh, 2015; Pelzer & Allan, 2012). Furthermore, antimicrobials agents in the culture medium provide little inhibition to the [potentially large number of bacteria (Moore, 2000). As well, the incidence of microbial infection after retrieval decreased from 0.4% to 0% in the patients that received antibiotics before IVF cycles (Gardner&Simón, 2016). In our study, we have determined the effects of seven different common antimicrobial agents including (CN, DOX, DA, MET, AMC, E, and FEP) on the bacterial isolates for antimicrobial susceptibility test using disk diffusion method. We found that most of the bacterial isolates showed high susceptibility 67% to AMC and FEP, moderate susceptibility 21% to E and 100% resistant to MET, then 58% to CN according to Clinical and Laboratory Standards Institute (Clinical Lab Standards Institute, 2016).

5. CONCLUSION

This study investigated the microorganisms within FF of women undergoing IVF procedure. No single species of bacteria tend to be associated with the IVF outcomes. Follicular fluid is not sterile but have no significant adverse effects of FF microbes on the IVF outcomes. Furthermore, microbiological analysis of FF samples for women undergoing IVF procedure may provide an opportunity to initiate antimicrobial treatment prior to the next conception.

Acknowledgment:

We would like to acknowledge Prof Hassan Abduljabar for providing the samples and Dr Azhar Najjar and Ibtesam Al Osaimy who helped with data Analysis.

Funding:

This study did not receive any external fund.

Conflict of Interest:

The author declare that there are no conflicts of interests.

Ethical approval:

The study was approved by the Biomedical Ethics Unit at King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Data and materials availability:

All data associated with this study are present in the paper.

REFERENCES AND NOTES

- World health organization. Multiple definitions of infertility. 2016; [Online] Available from: <https://www.who.int>
- Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of human reproductive sciences* 2015; 8(4):191.
- Anwar S, Anwar A. Infertility: A review on causes, treatment and management. *Women's Health Gynecol* 2016; 5(6):2.
- Mascarenhas MN, et al,. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS medicine* 2012; 9(12):e1001356.
- Al-Turki HA. Prevalence of primary and secondary infertility from tertiary center in eastern Saudi Arabia. *Middle East Fertility Society Journal* 2015; 20(4):237-240.
- Homan G, Davies M, and Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Human reproduction update* 2007; 13(3):209-223.
- Aoun TB, Moawed S. Effects of environmental, cultural, and socioeconomic factors on Saudi infertile couple in Riyadh City. *Life Science Journal* 2012; 9(4):4861-4868.
- Pelzer ES, et al,. Microbial colonization of follicular fluid: alterations in cytokine expression and adverse assisted reproduction technology outcomes. *Human reproduction* 2011 26(7):1799-1812.
- Pomeroy K. Contamination of human IVF cultures by microorganisms: a review. *J Clin Embryo* 2010; 13(4):11-30.
- Basuino L, Silveira JC, Human follicular fluid and effects on reproduction. *JBRA assisted reproduction* 2016; 20(1):38-40.
- Revelli A, et al,. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reproductive biology and endocrinology* 2009; 7(1):40.
- Borges E, Vireque A. Microbial Contamination in Assisted Reproductive Technology: Source, Prevalence, and Cost. *Advances in Biotechnology & Microbiology*, 2019; 13(4):555869.
- Pelzer ES, et al,. Microorganisms within human follicular fluid: effects on IVF. *PloS one* 2013; 8(3): e59062.
- Pelzer ES and Allan JA. The isolation and identification of microorganisms in the reproductive environment: the potential impact on the IVF culture system and on IVF outcomes. *Journal of Clinical Embryology* 2012; 15(3):44-53.
- Moore D, et al,. Bacteria in the transfer catheter tip influence the live-birth rate after in vitro fertilization. *FertilSteril* 2000 ;74(6):1118-24.
- Hamad TA, et al,. Microbial colonization of human follicular fluid and adverse outcome on in vitro fertilization cases in Kamal al-Samarrai's Hospital for fertility and In vitro fertilization treatment in Baghdad, Iraq. *Dental and Medical Sciences* 2018; 17(5):80-87.
- Ibadin KO, Osemwenkha AP. Microbiological study of infertile women programmed for invitro fertilization-embryo transfer in a tertiary health institution In Nigeria. *International Journal of Microbiology and Immunology Research* 2014; 3(1):6-11.
- Kim S, et al,. The incidence of positive bacterial colonization in human follicular fluids and its impact on clinical in vitro fertilization outcomes. *Fertility and Sterility* 2018;110(4):e149.
- CarterGR, Cole Jr, Diagnostic procedure in veterinary bacteriology and mycology. Fifth edition. 2012; Academic Press, Inc.
- Pelzer ES. Microbial colonisation of human follicular fluid and adverse in vitro fertilisation outcomes. 2011; Doctoral dissertation, Queensland University of Technology.
- Hemraj V, DikshaS, G Avneet. A review on commonly used biochemical test for bacteria. *Innovare J Life Sci* 2013; 1(1):1-7.

22. Bergey DH, et al., Bergey's manual of systematic bacteriology Archaea and the deeply branching and phototrophic bacteria. Vol. 1. 2001; Springer.
23. Alnefaie Z, MH Mutawakil, Ahmed MM. Molecular Identification for Camel Udder Microbiota. *Advances in Environmental Biology* 2019; 13(4):7-13.
24. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. 2009.
25. CLSI, Performance standards for antimicrobial susceptibility testing. Clinical Lab Standards Institute 2016.
26. Vos P, et al., Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes. Vol. 3. Springer Science & Business Media.
27. Pelzer ES, et al., TUNEL analysis of DNA fragmentation in mouse unfertilized oocytes: the effect of microorganisms within human follicular fluid collected during IVF cycles. *Journal of reproductive immunology* 2013; 99(1-2):69-79.
28. Aleshkin VA, Voropaeva EA, Shenderov BA. Vaginal microbiota in healthy women and patients with bacterial vaginosis and nonspecific vaginitis. *Microbial ecology in health and disease* 2006; 18(2): 71-74.
29. Bernard K. The genus *Corynebacterium* and other medically relevant coryneform-like bacteria. *Journal of clinical microbiology* 2012;50(10): 3152-3158.
30. Matytsina L, Greydanus D, Gurkin Y. Vaginal microbiocenosis and cytology of prepubertal and adolescent girls: their role in health and disease. *World Journal of Pediatrics* 2010; 6(1): 32-37.
31. Donati L, et al., Vaginal microbial flora and outcome of pregnancy. *Archives of gynecology and obstetrics* 2010; 281(4):589-600.
32. Stoll BJ, et al., Early onset neonatal sepsis: the burden of group B Streptococcal and *E. coli* disease continues. *Pediatrics* 2011; 127(5):817-826.
33. Chen L, et al., Rapid Sanger sequencing of the 16S rRNA gene for identification of some common pathogens. *PloS one* 2014; 9(2).
34. Gardner DK and Simón C. Handbook of in vitro fertilization. 2017; CRC press.