The impact of gram positive bacteria on semen quality in infertile men and assessment antibiotic Susceptibility profile

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Abstract

Despite of bacterial infections have been known as a potential condition of male infertility but clear definition and role of these conditions is not very clear. Presence of bacteria in the genital tract has been frequently discovered to be associated with reduced sperm function and as a result causes infertility. The goal of this study was to identify bacterial pathogens in infertile men's sperm culture and their antibiotic susceptibility patterns in vitro, which would aid in the formulation and monitoring of antibiotic policies and appropriate empiric therapy. A total of 50 semen specimen were collected from infertile men and 50 samples from healthy individual as control group were attending Kamal Al-Sammrai Hospital / Baghdad during the period 25 May 2020 to 15 October 2020.Seminal bacterial diagnosis were first done by the manual culture methods and confirmation by Vitek 2 system. The current study shown that, 76% isolates were gram positive bacteria and 24% was no growth of bacteria. *Staphylococcus warneri* (20%) followed by *Staphylococcus aureus* (18%), *Staphylococcus haemolyticus* (14%), *Staphylococcus saprophyticus* (12%), *Staphylococcus lentus* (6%), *Enterococcus faecalis* (4%) and *Streptococcus agalactiae* (1%). This study indicated that some of gram positive bacteria may be causes poor health of seminal fluid. Record some of *Staphylococcus* species were sensitive to Nitrofurantoin and Rifampicin while Moxifloxacin, Teichoplanin and Fusidic acid

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resistant from most *Staphylococcus* species. *Enterococcus faecalis* and *Streptococcus agalactia* were senetive to Rifampicin while have high resistance aganist Moxifloxacin, Nitrofurantoin and Fusidicacid.Because sperm bacterial infection is common and can cause sperm quality to deteriorate in infertile men, seminal fluid testing for bacterial detection should be done on a regular basis.

Keywords: *Gram positive bacteria, Male infertility, Antibiotic susceptibility*.

تأثير البكتيريا موجبة الجرام على جودة السائل المنوي لدى الرجال المصابين بالعقم وتقييم حساسية المضادات الحيوية

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الخلاصة

على الرغم من أن الالتهابات البكتيرية معروفة بأنها حالة محتملة لعقم الذكور ، إلا أن التعريف الواضح لهذه الحالات ودورها ليس واضحًا تمامًا. تم اكتشاف وجود بكتيريا في الجهاز التناسلي بشكل متكرر على أنه مرتبط بضعف وظيفة الحيوانات المنوية ونتيجة لذلك يسبب العقم. كان الهدف من هذه الدراسة هو التعرف على مسببات الأمراض البكتيرية في زراعة الحيوانات المنوية للرجال المصابين بالعقم وأنماط الحساسية للمضادات الحيوية في المختبر ، والتي من شأنها أن تساعد في صياغة ومراقبة سياسات المضادات الحيوية والعلاج التجريبي المناسب. تم جمع 50 عينة من السائل المنوي من رجال مصابين بالعقم و 50 عينة من الأفراد الأصحاء كمجموعة سيطرة الوافدين الى مستشفى كمال السامرائي / بغداد خلال الفترة من 25 مايو 2020 إلى 15 أكتوبر 2020 ، وتم التشخيص البكتيري النموي بوساطة طرق الاستزراع أولا والتأكيد بواسطة نظام الفايتك ثانيا. اظهرت الدراسة الحالية ان 76% من العزلات كانت بكتريا موجبة لصبغة غرام و24% لا يوجد بها نمو.

Staphylococcus warneri (20% تليها *Staphylococcus aureus* (18٪ ،) *Staphylococcus haemolyticus* (14٪ ،) *Staphylococcus saprophyticus* (12٪ ،) *Staphylococcus lentus* (6٪ ،) *Enterococcus faecalis* (4%) و *Streptococcus agalactiae* (1%) . أشارت هذه الدراسة إلى أن بعض البكتيريا موجبة الجرام قد تكون سببًا في تدهور السائل المنوي. سجل أن بعض أنواع المكورات العنقودية كانت حساسة للنيتروفورانتوين والريفامبيسين بينما

مقاومة من معظم المكورات العنقودية. Moxifloxacin, Teichoplanin and Fusidicacid
كانت حساسة للريفامبسين بينما كانت عالية *Enterococcus faecalis*و *Streptococcus agalactia*
المقاومة ضد كل من Moxifloxacin, Nitrofurantoinو Fusidicacid.

لأن العدوى البكتيرية للحيوانات المنوية شائعة ويمكن أن تتسبب في تدهور جودة الحيوانات المنوية لدى الرجال المصابين بالعقم ، لذالك يجب إجراء اختبار السائل المنوي للكشف عن البكتيريا بشكل منتظم.

الكلمات المفتاحية : *بكتريا موجبة لصبغة غرام , عقم الرجال , حساسية المضادات الحياتية*

1.Introduction

Infertility is a health issue that affects roughly 10% of the world's population. This word refers to a couple's failure to conceive after a year of regular unprotected sexual contact. Which happens in 80-85 percent of couples without determinants after 12 months [1]. Infertility affects 13-15 percent of couples, and the male component is directly or indirectly responsible for 60% of these infertile couples [2]. Abnormal fertility process through the following mechanisms: spermatogenesis deterioration, decreased sperm motility, altered acrosome reaction, altered morphology, formation of reactive oxygen species leading to increased DNA fragmentation index, formation of antisperm antibodies due to a breach in the blood–testes barrier, and genital tract obstruction due to inflammation and fibrosis [3].

Several factors can trigger inflammation, including accessory gland malfunction, oxidative stress, structural obstacles in the seminal tube, and microorganism infections that directly alter semen quality [4]. Bacterial invasion of the male reproductive tract damages spermatozoa and contributes to sperm quality degradation by colonizing and contaminating the male urogenital tract [5]. According to a number of studies [6,7,8], infections and inflammations of the male genitourinary tract produced by pathogenic microorganisms such as bacteria, virus, fungus, and protozoa are associated to 8-32 percent of male infertility cases. Infections with these pathogenic agent’s cause infertility issues such as sperm damage, pyospermia,

asthenospermia, and teratospermia by impairing spermatogenesis, resulting in inflammatory disorders, anatomical obstruction, scarring, and the activation of the leukocyte response, which causes oxidative stress [9]. Leukocytospermia can be caused by a variety of factors, including environmental pollutants, vaginal items used during intercourse, alcohol, cigarettes, some drugs, and surgical manipulation [10].

In subfertile men, however, there appears to be a link between bacteriospermia and leukocytospermia. Infection of the testis, epididymis, and prostate in the male genitourinary system can impair spermatogenesis and reproductive potential [11]. The purpose of this study was to determine the prevalence of bacterial flora in sperm and its relationship to sperm quality and sperm characteristics in infertile males.

2.Materials and Methods

2.1. Seminal fluid collection

During the period of 25 May 2020 to 15 October 2020, fifty semen specimens were obtained from infertile men and fifty semen specimens were collected as study controls from fertile men who visited Kamal Al-Samarrai Hospital for Infertility and in Vitro Fertilization Infants in Baghdad. After 3–7 days of sexual abstinence, semen specimens were collected by masturbating into glass with wide-mouth or plastic containers given by the laboratory. The sample was sent directly to the research lab and kept in an incubator at 37 degrees Celsius until it liquefied completely.

2.2. General seminal analysis

The pH, volume, presence of pus/immature cells, sperm motility, sperm concentration, and normal / aberrant morphology of the sperm were all assessed according to WHO criteria [1].

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2.3. Semen culture

After ejaculation, seminal fluid specimens were placed on the workbench for 30 minutes to dissolve before being delivered. Chocolate agar, Blood agar, MacConkey agar, and mannitol agar were among the solid media used to inoculate 0.1ml of the specimen for regular laboratory cultures. The regular media were incubated for 24 hours in an aerobic atmosphere at 37C°, while chocolate agar was incubated in an anaerobic jar at the same temperature [12].

2.4. Vitek 2 compact system

Vitek 2 compact is a small, pre-programmed device that addresses microbial proof of identification and antimicrobial susceptibility testing (AST) by reducing performance time and allowing for faster recording. Although initial organism isolation is required, the TAT is 2 to 18 hours. Vitek 2 compact is a space-saving and cost-effective system. The idea of Vitek 2 compact's technology is based on a fluorogenic methodology for organism documentation and a turbidimetric system for antimicrobial susceptibility testing.

3. Statistical Analysis

The data was analyzed using the statistical package for the social sciences (SPSS) version 24. The Chi-square test was performed to assess the percentage (0.05 and 0.01) of likelihood, and the one-sample T-test was employed to compare means.

3. Results

The distribution of organism's species diagnosed by Vitek 2 Compact method showed in table (1). A total of 50 semen specimen, 38 (76%) were positive culture and 12 (24 %) no growth by classical culture method. From 38 (76%) positive culture, 38

(76%) showed significant Gram positive cocci by vitek 2 compact. was *Staphylococcus warneri* the most frequent with occurrence 10(20%) followed by *Staphylococcus aureus* 9(18%), *Staphylococcus haemolyticus* 7(14%), *Staphylococcus saprophyticus* 6(12*%)*, *Staphylococcus lentus* 3(6%), *Enterococcus faecalis* at a percentage 2(4%), finally 1 (2%) for *Streptococcus agalactiae*.

Table (1): Distribution of organisms in Semen according to their species by Vitek 2 Compact method

Organisms by vitek 2 compact	**Number (50)**	**Percentage% 100.00%**
Gram positive cocci	38	76.00%
Staphylococcus warneri	10	20.00%
Staphylococcus aureus	9	18.00%
Staphylococcus haemolyticus	7	14.00%
Staphylococcus saprophyticus	6	12.00%
Staphylococcus lentus	3	6.00%
Enterococcus faecalis	2	4.00%
Streptococcus agalactiae	1	2.00%
No growth	12	24.00%

The results in Table (2) showed that the comparison of semen volume and semen pH according to the results of bacteriological culture, there was no significant difference in semen volume $p \geq 0.05$ between *S. warneri*, *S. aureus*, *S. haemolyticus*, *S. saprophyticus*, *S. lentus*, and *E. faecalis* bacterial semen infection and control group, p value =0.8, p value =0.8, p value = 0.1, p value =0.5, p value =0.5 and p value = 0.1, respectively. Regarding to semen pH, in the present study, *S. warneri*, *S. aureus*, *S. haemolyticus*, *S. saprophyticus*, *S. lentus* and *E. faecalis* have no significant difference p value =0.1, p value =0.6, p value =0.1, p value =0.4, p value =0.4 and p value =0.5, respectively compare with control group and also no significant according to WHO, (2010). It is noteworthy *S. agalactiae* was small sample size which cannot show the effects on semen volume and pH.

Table (2) : Comparisions the influence of Gram positive bacteria on seminal fluid physical parameter**s**

Parameter / Type of bacteria	Volume/Ml N (1.5ml-6ml)	pH N (7.2-8.0)
	Mean±SD	Mean±SD
Control	2.41±0.82	7.48±0.85
S. warneri	1.31±0.98 *	7.54±0.09 *
S. aureus	1.72±1.03 *	7.57±0.13 *
S. haemolyticus	1.71±1.49 *	7.71±0.10 *
S.saprophyticus	1.50±1.00 *	7.61±0.22 *
S. lentus	2.16±0.76 *	7.66±0.11*
E. faecalis	1.75±0.35 *	7.35±0.21 *
S. agalactiae	3.0±0	7.60±0

***(non-significant)**

Table (3) shows the effects of isolated gram positive bacteria on semen count as well as (progressive motility, total motility, and dead cells). There was statistical reduction in the mean of sperm count (million/ ml) in groups *S. saprophyticus* (10.66±9.130) were p value P≤0.05. In this study, show high significant difference in sperm count in infected patients with *S. aureus, S. haemolyticus* and *S. lentus* were p value ≤0.01. While *S. warneri* and *E. faecalis* have no significant were p ≥ 0.05 comapre with control group. All isolated bacteria have no significant difference except *S. saprophyticus* (10.66±9.130) in semen count according to WHO, (2010). Regarding sperm motility. there was statstically significant of progressive motility P≤0.01 in *S. saprophyticus* and *S. lentus*, P≤0.001 in *S. warneri* and *S. haemolyticus* compare to control group. While *S. aureus* and *E. faecalis* have no significant difference p ≥ 0.05

in progressive motility infected patients. According to WHO, (2010), all isolated bacteria exhibit a considerable variance in progressive motility. Regarding total motility there were statistical difference P≤0.01 in patient infected with *S. aureus* compare with control group. While *S. warneri, S. haemolyticus, S. saprophyticus, S. lentus* and *E. faecalis* have no statistical difference p≥0.05 compare to contorl group. While regarding dead cells appear statistical difference P≤0.01among infertile men infected with *S. warneri*. Dead cells of *S. aureus* show high significant P≤0.001 in infertile patients compare to control group. Whereas *S. haemolyticus, S.saprophyticus, S. lentus* and *E. faecalis* were no significant difference p≥0.05 compare to control. *S. haemolyticus, S.saprophyticus* have high rate of dead cells compare to WHO, (2010). It is noteworthy *S. agalactiae* was small sample size which cannot shows the effects sperm count and motility.

Table (3) : Comparisons the influence of gram positive bacteria on sperm count and motility

Parameter / **Type of bacteria**	**Count million sperm/ml** **N(15Million sperm/ml)**	**progressive moyility** **N (32% or more)**	**Total motility** **N (at least 40%)**	**Dead cells** **N (Not more than 60%)**
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	46.54±19.38	20.37±9.60	56.70±13.82	43.30±13.82
S. warneri	24.2±22.26 *	0.50±1.58 c	32.50±21.11 *	47.50±27.91b
S. aureus	34.94±48.4 b	3.88±6.97 *	35.5±28.55 c	42.22±31.33 c
S. haemolyticus	36.42±35.75 b	0.0±0 c	34.28±19.66 *	65.71±19.66 *
S.saprophyticus	10.66±9.130 a	0.0±0 b	29.16±15.94 *	70.83±15.94 *
S. lentus	79.33±24.17 b	0.0±0 b	41.66±12.58 *	55.0±13.22 *
E. faecalis	76.5±27.57 *	5.0±7.07 *	67.5±10.60 *	32.50±10.60 *
S. agalactiae	35.0±0	0.0±0	50.0±0	50.0±0

***(non-significant), a(P≤0.05), b(P≤0.01), c(P≤0.001). Diverse litters=significant difference**

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The results in table (4) showed comparison in mean percentage of abnormal, normal sperm morphology according to gram positive bacterial isolated, *S. warneri* and *S. aureus* show statistical difference P≤0.01 between abnormal morphology of sperm and control group. Whereas *S. haemolyticus*, *S. saprophyticus*, *S. lentus* and *E. faecalis* have no statstical difference of abnormal morphology of sperm p≥0.05 compare to control group. *S. warneri* and *S. aureus, S. haemolyticus, S.saprophyticus, S. lentus* and *E. faecalis* were none statistically significant p≥0.05 compare to control group. However, *E. faecalis* have normal ratio of normal cells according to WHO, (2010). While other bacteria *S. warneri* and *S. aureus, S. haemolyticus, S.saprophyticus, S. lentus* and *E. faecalis* have lower number of normal cells in contrast with WHO, (2010). It is noteworthy *S. agalactiae* was small sample size which cannot shows the effects sperm count and motility.

Table (4): Comparisons the influence of gram positive bacteria on abnormal and normal sperm

Parameters / **Types of bacteria**	**Abnormal**	**Normal N (30% or more)**
	Mean ± SD	Mean ± SD
Control	38.60±16.15	61.40±2.28
S. warneri	60.5±33.86 b	19.5±15.35 *
S. aureus	52.22±31.43 b	25.55±17.93 *
S. haemolyticus	75.71±12.05 *	24.28±12.05 *
S.saprophyticus	74.16±9.70 *	25.83±9.70 *
S. lentus	71.66±2.88 *	28.33±2.88 *
E. faecalis	55.0±7.07 *	45.0±7.07 *
S. agalactiae	75.0±0	25.0±0

***(non-significant), b(P≤0.01),**

The relative between percentage of pus cells, immature cells and gram positive isolated bacteria was illustrated in table (5). The study detected that *S. warneri, S. aureus, S. haemolyticus* and *S.saprophyticus* have high statistical difference P≤0.001of immature cells compare to control group. However, there were significant difference

according to WHO, (2010). While *S. lentus* and *E. faecalis* have no significant difference p≥0.05 compare to control group. All isolated bacteria have low number of immature cells in contrast with WHO, (2010). Regarding of pus cells there were statstical differeance P≤0.001of pus cells infected group with *S. warneri*, *S. haemolyticus*, *S.saprophyticus* and *S. lentus* compare to control group.While *S. aureus* and *E. faecalis* have no significant differeance p ≥0.05 comapre to control group. All isolated bacteria have high significant in contrast with WHO, (2010). It is noteworthy *S. agalactiae* was small sample size which cannot shows the effects immature cells and pus cells.

Table (5): Comparisons the influence of gram positive bacteria on immature cells and pus cells

Parameters / **Types of bacteria**	**Immature cells** **N (<5 HPF)** Mean ± SD	**Pus cells** **N (≥1 × 10^6/mL** Mean ± SD
Control	0.76±0.79	0.52±0.76
S. warneri	2.20±2.34 c	7.60±3.74 c
S. aureus	2.22±3.34 c	5.55±1.13 *
S. haemolyticus	2.57±2.22 c	7.0±2.38 c
S.saprophyticus	1.0±2.44 c	6.0±3.09 c
S. lentus	1.66±1.52 *	7.33±4.04 c
E. faecalis	1.0±1.41 *	7.0±1.41 *
S. agalactiae	0.0±0	6.0±0

***(non-significant), c(P≤0.001)**

Table (6. A1) show the Antibiotic susceptibility patterns against *S. warneri*, *S. aureus* isolated from semen specimens. The current investigation has shown antibiotics resistance and sensitivity rates against semen-isolated bacteria. *S. warneri* had the highest sensitivity rates to Nitrofurnation, Rifampicin, and Vancomycin, with 80.0 %,

60.0 %, and 20.0 %, respectively. While *S. aureus* (77.8%), (66.7%) exhibit the high rate of sensitivity to Nitrofurnation and Rifampicin respectively. The results revealed that *S. warneri* showed high resistance (70.0%) to Fusidicacid and (70.0%) to Teichoplanin. The highest rate of *S. aureus* resistance to Moxifloxacin was (77.8%) and Fusidic acid was (66.7%).

Table (6. A1): Antibiotic Susceptibility (%) patterns of Gram positive bacteria in semen

Organisms	***S. warneri (N=10)***				***S. aureus (N=9)***			
Antibiotics	N%	R%	S%	I%	N%	R%	S%	I %
Benzylpencillin	80.0	20.0	-		55.6	44.4	-	
Oxacillin	80.0	20.0	-		55.6	44.4	-	
Gentamicin	60.0	30.0	1.0		77.8	22.2	-	
Tobramycin	70.0	20.0	1.0		55.6	22.2	22.2	
Levofloxacin	60.0	40.0	-		33.3	55.6	11.1	
Moxifloxacin	30.0	60.0	1.0		22.2	77.8	-	
Erythromycin	80.0	20.0	-		44.4	55.6	55.6	
Clindamycin	80.0	20.0	-		44.4	-	55.6	
Linezolid	80.0	20.0	-		44.4	-	55.6	
Teichoplanin	30.0	70.0	-		22.2	44.4	-	33.3
Vancomycin	80.0		20.0		44.4	-	55.6	
Tetracycline	80.0	20.0			44.4	55.6	-	
Nitrofurantion		10.0	80.0	10.0		-	77.8	22.2
Fusidic acid	30.0	70.0	-		33.3	66.7	-	
Rifampicin	30.0	1.0	60.0		33.3		66.7	
Trimmethoprim/ sulfamethoxazole	50.0	40.0	1.0		77.8	22.2		

N: Not tested, S: Sensitivity, R: Resistance, I: Intermediate

Antibiotic susceptibility patterns against *S. saprophyticus*, *S. haemolyticus*, and *S. lentus* isolated from semen specimens are shown in Table 6. A2. *S. saprophyticus* (100.0%), (50.0%), and *S. haemolyticus* (70.0%), (57.1%) have high rates of sensitivity to Nitrofurantoin and Rifampicin, respectively, according to the current

investigation. Rifampicin and Gentamicin sensitivity is low in *S. lentus* (33.3 %). *S. saprophyticus* was shown to be resistant to Fusidic acid, Teichoplanin, and Moxifloxacin in 66.7%, 50.0 % and 50.0 % of cases, respectively. The rate of resistance to Moxifloxacin and Trimethoprim/sulfamethoxazole by *S. haemolyticus* (71.40%). *S. lentus* had low resistance to Levofloxacin (33.3%), Moxifloxacin (33.3%), Teichoplanin (33.3%), and Fusidic acid (33.3%).

Table (6. A2): Antibiotic Susceptibility (%) patterns of Gram positive bacteria in Semen

Organisms	***S. saprophyticus* (N=6)**				***S. haemolyticus* (N=7)**				***S. lentus* (N=3)**		
Antibiotics	N%	R%	S%	I %	N%	R %	S%	I%	N%	R%	S%
Benzylpencillin	66.7	33.3	-		57.1	42.9	-		100	-	-
Oxacillin	66.7	33.3	-		42.9	57.1	-		100	-	-
Gentamicin	50.0	16.7	33.3		14.3	57.1	28.6		66.7		33.3
Tobramycin	66.7	33.3	-		57.1	28.6	14.3		100	-	-
Levofloxacin	66.7	33.3	-		42.9	42.9	14.3		66.7	33.3	-
Moxifloxacin	33.3	50.0	16.7		28.6	71.40	-		66.7	33.3	-
Erythromycin	66.7	33.3	-		57.1	42.9	-		100	-	-
Clindamycin	66.7	16.7	16.7		42.9	14.3	42.9		100	-	-
Linezolid	66.7	-	33.3		42.9	-	57.1		100	-	-
Teichoplanin	33.3	50.0	-	16.7	42.9	14.3	-	42.9	66.7	33.3	-
Vancomycin	66.7	-	33.3		42.9	14.3	42.9		100	-	-
Tetracycline	66.7	33.3	-		42.9	57.1	-		100	-	-

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Nitrofurantion	-	-	100		-	-	70.0		100	-	-
Fusidicacid	33.3	66.7	-		42.9	57.1	-		66.7	33.3	-
Rifampicin	33.3	16.7	50.0		42.9	-	57.1		66.7	-	33.3
Trimmethoprim /sulfamethoxazole	50.0	16.7	33.3		14.3	71.40	14.3		100	-	-

N: Not tested, S: Sensitivity, R: Resistance, I: Intermediate

Table (6. B) show the susceptibility patterns of *Enterococcus feacalis* and *S. agalactia* organism in semen. *Enterococcus feacalis* has a sensitivity rate (50.0%) to Vancomycin and (50.0%) to Rifampicin. While *S. agalactia* has high sensitivity rate (100.0%) to Rifampicin. *Enterococcus feacalis* has high resistance rate (100.0%) to Moxifloxacin, (100.0%) to Nitrofurantoin and (100.0%) to Fusidicacid and *S. agalactia* has high resistance rate (100.0%) to each of Moxifloxacin, (100.0%) to Teichoplanin, (100.0%) to Nitrofurantoin and (100.0%) to Fusidicacid.

Table (6. B): Antibiotic Susceptibility (%) patterns of Gram positive bacteria in Semen

Organisms	***Enterococcus faecalis (N=2)***				***S.agalactia* (N=15)**		
Antibiotic	N%	R%	S%	I%	N%	R%	S%
Benzylpencillin	50.0	50.0	-		100.0	-	-
Oxacillin	50.0	50.0	-		100.0	-	-
Gentamicin	100.0	-	-		100.0	-	-
Tobramycin	50.0	50.0	-		100.0	-	-
Levofloxacin	100.0	-	-		100.0	-	-

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Moxifloxacin	-	100.0	-		-	100.0	-
Erythromycin	50.0	50.0	-		100.0	-	-
Clindamycin	50.0	50.0	-		100.0	-	-
Linezolid	100.0	-	-		100.0	-	-
Teichoplanin	50.0		-	50.0	-	100.0	-
Vancomycin	50.0	-	50.0		100.0	-	-
Tetracycline	50.0	50.0	-		100.0	-	-
Nitrofurantoin	-	100.0	-		-	100.0	-
Fusidicacid	-	100.0	-		-	100.0	-
Rifampicin		-	50.0	50.0	-	-	100.0
Trimmethoprim/ sulfamethoxazole	100.0	-	-		100.0	-	-

N: Not tested, S: Sensitivity, R: Resistance, I: Intermediate

Discussion

Bacterial invasion of the male reproductive system harms spermatozoa and plays a role in lowering sperm quality by colonizing and polluting the male urogenital tract. This is the most contentious subject [13]. Microorganisms can impair male reproductive function directly by inducing motile sperm agglutination, limiting the ability of acrosome reactions and altering cell shape, or indirectly by producing reactive oxygen species as a result of the inflammatory response to infection [14,15]. Present study indicated that *S. warneri* was the most frequent organisms followed by *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*,

Enterococcus faecalis and *Streptococcus agalactiae* as shown in Table (1). The current study no agreement with the study done by Sasikumarm, *et al*. [16] showed the most predominant bacteria is *Staphylococcus aureus* (43.33%).

Also Khadim and Al- Bermani [17] disagreement with current study detected *Streptococcus thoracentesis* (17.1%), *Staphylococcus aureus* (15.7%) and *Enterococcus faecalis* (5.7%). The reason for this diversity may be due to different in technique used to diagnose the bacteria. [18] exhibited the percentage of bacteria isolated from semen specimen by vietk 2 compact technequie were 14 (17.5%) *Staphylococcus haemolyticus* and 2 (4%) *Enterococcus faecium*, which no consistent with present study. In Table 2 shows the influence of gram negative bacteria on semen pH and volume. Because of its low sensitivity and specificity, semen pH cannot be used as a diagnostic tool to distinguish infected from non-infected persons [15]. In terms of semen volume, the current study agreed with Karthikeya, *et al*., [19] who found that the average semen volume was 2 ml. Sperm concentration is another sperm characteristic that is important in male infertility [20]. The current study's findings as shown in Table (3) were similar with the study implemented by Khadim and Al-Bermani [17] were showed a significant difference represented by decrease in the concentration of sperm ($P \leq 0.05$) between bacterial infected semen samples when compared with control group. [21] found that bacteriospermia was connected with sperm quality, particularly sperm count. Regarding of sperm motility, the current study agreement with Fatemeh, *et al*. [22] showed in your study a loss of motility in seminal bacterial contamination, especially for *S. aureus*, and *S. haemolyticus*, similary to our study where each of *S. aureus*, and *S. haemolyticus* show reduction in total motility. On the other hand , [23, 1, 24] found that sperm concentration, morphology, and motility were not significantly affected in bacteriospermic specimens.

A seen in table (4), gram positive bacteria have a poor influence on normal sperm. The results of this study correspond with those of Al-Saadi and Abd [25], who

found a decrease in the number of sperm with normal morphology in the *Staphylococcus* infected group. One the other hand, study done by Merino, *et al*., [26] record no decrease in number of normal sperm of infected group.

This variation could be related to the diverse bacterial species identified, which have varied effects on sperm morphology. The relative between percentage of pus cells, immature cells and gram positive isolated bacteria was illustrated in table (5). Number of studies agreement with present study, among of them study done by Domes, *et al*. [10] found that the presence of leukocyte in semen specimens, with or without bacteriospermia, had a negative impact on semen quality, including sperm concentration, motility, and morphology. Leukocytospermia is an inflammatory condition possibly attributed to inflammation or infection in the semen [27]. In contrast, a number of studies have found no statistically significant link between leukocytospermia and bacteriospermia in ejaculated sperm [13]. The present study disagreement with the result of [25] which found *Staphylococcus* have highest number of immature cells (15.57 ± 8.94).Numerous research across the world have studied antibacterial sensitivity tests, but relatively few about seminal fluid-isolated microorganisms, therefore the current study revealed Gram positive bacteria's antibiotic susceptibility patterns in sperm as shown in Table (6. A1, A2). This study agreement with Hathiwala, *et al*., [28] study which found that most of the Gram positive cocci were sensitive to Linezolid, Vancomycin, and Nitrofurantoin, at the same time this study disagrees with regard Gram positive cocci sensitivity to Teichoplanin where present study found *Staphylococcus* species resistance at different rates to Teichoplanin. Another study by Bakhtiari, *et al*., [29] recorded, similarly *S. aureus* was found (81.83%) sensitive to Nitrofurantoin. On the other hand, study by Nasrallah, *et al*., [30] not agreeable with present study were found gram-positive bacteria (*S. aureus*, *Streptococcus* spp., and Coagulase-Negative *Staphylococcus*) are

highly sensitive to linezolid, vancomycin, azithromycin, clindamycin, Teichoplanin, erythromycin, and azithromycin.

The majority of Gram-positive bacterial isolates had higher sensitivity patterns to vancomycine, daptomycin, nitrofurantoin, gentamicin, and linezolid, with sensitivity rates of 100%, 98.1%, 97.1%, 93.0%, and 92.10%, respectively Bitew, *et al*., [31] which disagreement with present study which appear that *Staphylococcus* species has sensitivity to Nitrofurnation, Rifampicin, Gentamicin and Vancomycin with a sensitivity rate (100.0%) in *S. saprophyticus*, (66.7%) in *S. aureus*, (33.3%) in *S. lentus* and (20.0%) in *S. warneri* respectively. Mogram *et al*., [32] disagreement with recent study which found *Streptococcus feacalis* was sensitive to trimethoprim-Sulphamethoxazole followed by nitrofurantoin and erythromycin whereas current study found *Enterococcus feacalis* sensitive to Vancomycin and Rifampicin as shown in Table (6. B). Bhatt, *et al*., [33] study no agreeable with present study which detected that *S. feacalis* had high rate of sensitivity (100.0%) for nitrofurantoin and (71.4%) for each of Gentamycin, levofloxacin and ampicillin-sulbactam and (100.0%) resistance to Cephalexin. Other study (Nasrallah, *et al*., [30] detected that *Streptococcus* spp. were highly sensitive to linezolid, vancomycin, azithromycin, clindamycin, teichoplanin and erythromycin whereas the result of present study shows *S. agalactia* high sensitivity to Rifampcin only.

Conclusion

1. Routine bacterial culture methods are still important in diagnosing bacteria, but they are insufficient in diagnosing bacterial species, which are diagnosed using the vitek 2 Compat, which expresses bacterium species based on biochemical assays integrated in an ID card.

2. Infertile males should have their sperm bacteriological cultured on a regular basis since bacteria might impact the semen parameters.

3. In order to treat an infection with appropriate medications, the antimicrobial susceptibility of the infecting bacteria must be established early in the infection process in order to create a unique treatment plan.

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