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Detection of Bacterial Isolates Associated with Semen Among Persons with Temporary Infertility in the City of Ramadi

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ABSTRACT

Background: Infertility is the inability to bear children after at least one year of unprotected intercourse. It is a health problem that affects about 10% of the world's population.

Aim of the work: This study aims to examine semen-associated bacterial isolates of people with temporary infertility and to determine the appropriate antibiotic.

Materials and Methods: Samples were collected from patients after they had abstained from sexual activity for 3-7 days before semen sample collection. Positive bacterial isolates were isolated for bacterial culture and growth on the surface of blood agar, MacConkey agar, or cooked blood agar and diagnosed according to standard and biochemical tests. The final diagnosis of bacterial isolates was performed using detection strips for Gram-positive and Gram-negative APIE20.

Results: The study showed that most isolates of Gram-positive bacteria were Staphylococcus aureus (25.97%), followed by Streptococcus agalactiae (18.93%). The study showed that Escherichia coli was the most prevalent among Gram-negative isolates (31.6%), followed by Klebsiella pneumoniae (8.97%), followed by Pseudomonas aeruginosa (3.39%). The study also showed that all bacterial isolates are sensitive to the antibiotics Levofloxacin, Meropenum, Doxycycline, and Amikacin, respectively.

Conclusion: The presence of bacterial isolates in the semen is a clear reason for temporary sterility and the need to treat them with antibiotics.

Keywords: Temporary Infertility , Seminal fluid , Bacterial , Semen infection , antibiotics.

INTRODUCTION

The World Health Organization defined *Infertility* as the inability to have children at least after one year of intercourse without protection. *Infertility* is a health problem affecting about 10% of the world's population [(1)]. There is disagreement about the effect of bacteria on male Infertility. However, many studies indicate that many bacterial species are associated with semen in infertile people and influence the chances of reproduction [(2)]. Semen is one of the sterile fluids in the human body, and 90% of the semen volume consists of a mixture of secretions from the epididymis, prostate, seminal vesicles, and glands surrounding the urethra [(3)].

The pH of semen ranges between (7.2-8) and is rich in fats, sugars, inorganic ions, organic components, enzymes, nucleic acids, proteins, and peptides, essential materials in creating suitable environments microorganisms grow [(4)]. The fructose sugar produced by the seminal vesicles provides energy for sperm metabolism and food for microorganisms if present [(5)]. The sources of semen contamination with bacteria originate from the urinary tract or may arise through sexual contact, so these pathogens are transmitted to the man. It is a problem for infertile people [(6)].

In a study, it was shown that bacteria cause inflammation of the lining of the seminiferous tubules, which leads to the activity of white blood cells to attack in one way or another the bacteria and some of the sperm[(7)], which may cause a decrease in the volume and density of the ejaculated semen and a lack of sperm motility [(8)]. The damage of bacteria may extend to the auxiliary gonads, including the seminal vesicle, causing a decrease in the volume of each ejaculate and a decrease in the proportion of fructose sugar in the plasma is necessary to supply the sperm with energy. Whether or not it was infected with bacteria [(9)].

The bacterial culture of semen is considered one of the most important diagnostic methods for detecting the extent of bacterial infection of semen. The presence of bacteria in semen at a concentration of 103/ml is a clinical sign of an infection called sperm bacteria [(10)]. Many previous studies showed that treating people with temporary Infertility with antibiotics increased fertility and chances of conception. It was concluded that bacteria were the cause of Infertility through their presence in semen[(11)]. Infertility rates vary significantly between countries of the world. Up to a third of couples experience Infertility, and approximately 52% suffer from acquired Infertility. On the contrary, the secondary infertility rate is lowest in Asia, at 23%, and in developed countries, at 29% [(12)].

A study showed that out of 120 semen samples collected from males admitted to infertility centers in Najaf Governorate, 74 samples (61.66%) showed positive growth of bacteria[(13)]. In another local study, (85%) of the semen samples contained bacterial isolates [(14)]. While in a study in Iran, (21%) of the samples contained bacteria in the samples [(15)].In a study at Shanghai University in China, 23.4% of semen samples

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contained multiple types of bacterial isolates [16]. While in a study in Nigeria showed that (18%) of the semen sample carried different types of bacterial isolates [17]. As for the study conducted by [18], the percentage of bacteria present in semen samples was (66.3%). In a study conducted at the Fancourt Hospital, a proportion (56%) of semen samples contained bacterial isolates [19].

MATERIALS AND METHODS

The current study was conducted in a group of external laboratories associated with some infertility doctors in Ramadi.

Sample collection

Six hundred fifty-six semen samples were collected from males who had temporary or permanent infertility in outpatient clinics for two years. The samples were collected from patients after they abstained from sexual activity for 3-7 days before the semen sample was taken. Personal hygiene was taken into account as requested by the patient. He urinates, cleans his penis with water, and washes his hands well before taking the sample. The semen was collected in a sterile plastic vial designated for collecting semen. Then the sample was transferred directly to the laboratory and incubated at 37 °C for liquefaction. Then mixed, the semen was with a saline solution and centrifuged for 15 minutes. The sediments were taken according to the instructions of the World Health Organization and planted on blood agar media, MacConkey agar medium, and cooked blood medium. The dishes were incubated aerobically and in the presence of CO_2 at 37°C for 24 hours to develop bacterial isolates associated with semen, if any.

Bacterial isolation and identification

Positive bacterial isolates for bacterial culture and growing on the surface of blood agar, MacConkey agar, or cooked blood agar were isolated and diagnosed according to standard and biochemical tests[20]. Then, the final diagnosis of bacterial isolates was carried out using API20E gram-positive and gram-negative bacteria detection strips supplied by the French company Biomerieux.

Detection of sensitivity to antibiotics

The antibiotic sensitivity test of bacteria isolated from semen was carried out according to the method prepared by clinical and laboratory standards using the Kirby-Bauer method[20]. The vaccine was prepared from growing bacterial isolates at 18 hours, then the density of each isolate was adjusted using a standard McFarland 0.5 test tube for growth turbidity. Then 100 µm of bacterial growth was taken and spread over the surface of Muller-Hinton agar using sterile cotton buds. The plates were left for a short period to dry. Then antibiotic tablets prepared by (Bioanalyse, Turkey) were placed on the surface of the agar and pressed against the surface of the medium with a sterile needle. Antibiotics included: Ciprofloxacin, Clarithromycin, Claforane, Rifadin, Garamycin, Meropenum, Amikacin, Levofloxacin, Ceftriaxone, Doxycycline, Norfloxacin, Cefixime, Ampiclox, Cephalothin, Cefalexin, Amoxicillin, Trimethoprim, Nalidellic acid, Azithromycin, Nitrofurantoin. After that, the dishes were incubated for 24 hours at 37°C, and the areas of inhibition of bacterial growth were read and compared with the percentages set by the World Health Organization for the diameters of inhibition [20].

Ethical approval:

The study was approved by the Anbar University Academic and Ethical Committee. For study acceptance, each patient signed informed written permission. This experiment was done in compliance with the World Medical Association's Code of Ethics (Declaration of Helsinki) for human studies.

Statistical analysis

The collected data were coded, processed, and analyzed using the SPSS (Statistical Package for Social Sciences) version 26 for Windows® (IBM SPSS Inc, Chicago, IL, USA). The Shapiro Walk test was used to determine if the data were normally distributed. Qualitative data were represented as frequencies and relative percentages.

Chi-square test (χ^2) to calculate the difference between two or more sets of quantitative and qualitative variables. P value <0.05 was considered significant.

RESULTS

Six hundred fifty-six semen samples were collected from temporary infertility people visiting doctors' clinics in Ramadi. 412 (62.80%) samples were positive for bacterial culture on culture media, while 244 (37.20%) samples were free of bacterial growth, as shown in figure (1).

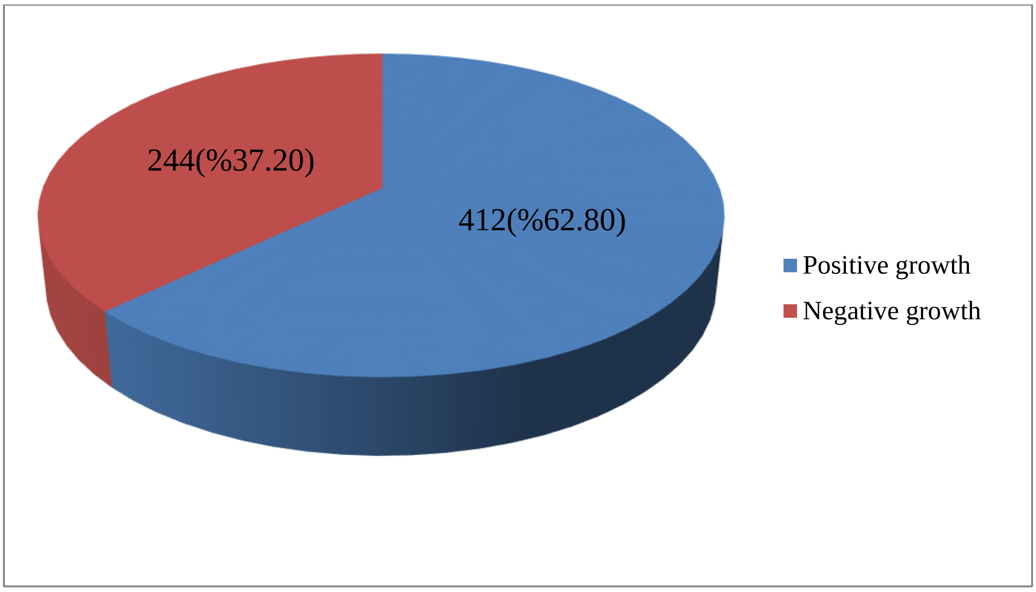

Fig. 1. Distribution of positive and negative samples for bacterial culture

The Positive samples for bacterial culture were taken for diagnosis and identification of bacterial species, taking into account the exclusion of duplicate patient isolates. The results showed that the age group that ranged between (31-40) years recorded the highest infection rate of 163 (39.56%), followed by the age group (18-30) years with 126 (30.58%), followed by the age group (18-30) years. The group (41-50) years old with a rate of 87 (21.11%), and the lowest level of infection was in the age group (51-60) years with a percentage of 38 (9.22%). The statistical results indicated a significant difference between age and number of isolates based on the (X^2) test at the probability level P < 0.05., as shown in Figure (2).

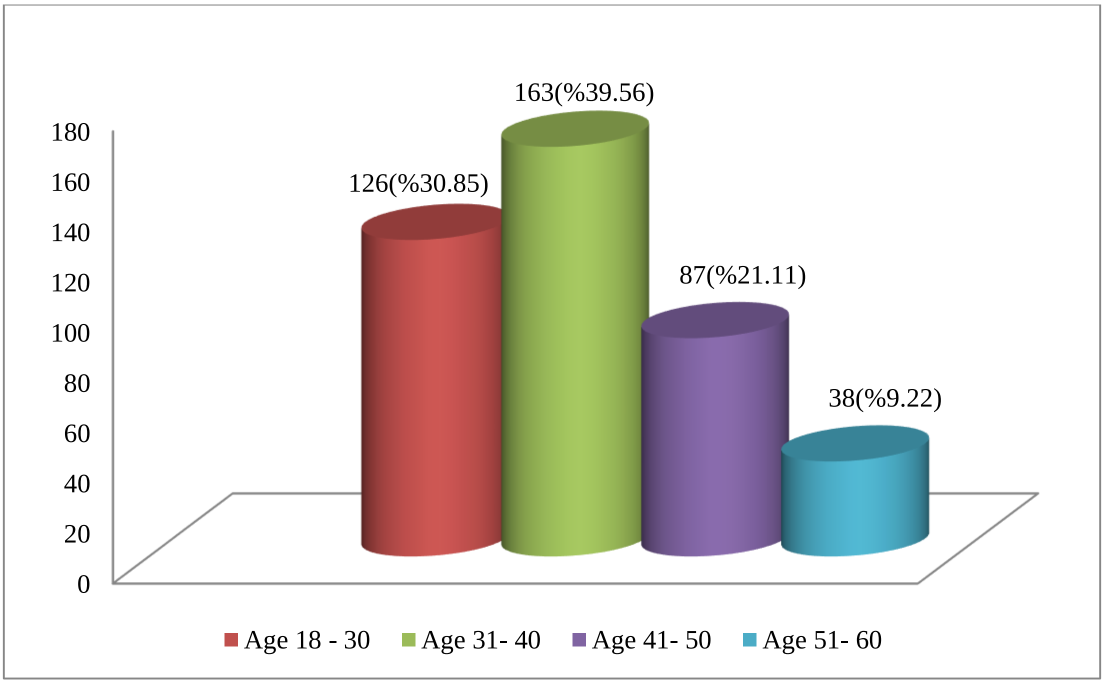

Fig.2. Distribution of positive semen samples for bacterial culture by age. There is a significant difference between age and number of isolates based on the X^2 test at the probability level P < 0.05.

The distribution of gram-positive and gram-negative bacteria accompanying the semen samples of the patients showed that the gram-positive bacteria had the highest percentage of the gram-negative bacteria with a percentage of 218 (52.91%), While the percentage of Gram-negative bacteria isolates was 186 (45.14%). In comparison, 8(1.98%) samples were mixed between Gram-positive and Gram-negative bacteria grown on one plate. It is difficult to isolate and differentiate them, as shown in Figure (3).

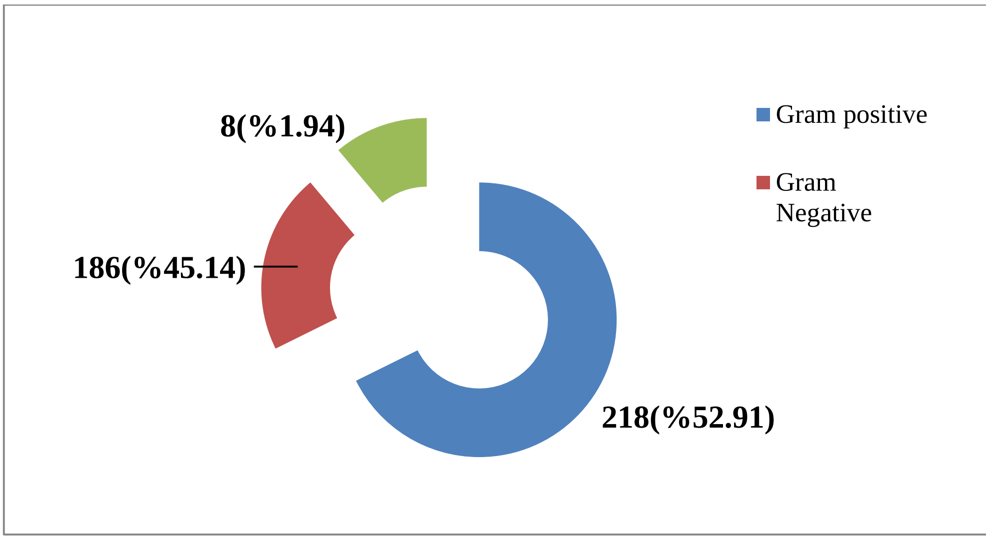

Fig.3. Distribution of Gram-positive and Gram-negative samples

The 218 gram-positive bacteria isolates were distributed to 107 (25.97%) of *Staphylococcus aureus*, which was more prevalent among the positive bacteria isolates, followed by *Streptococcus agalactiae* isolates with a rate of 78 (18.93 percent) In contrast, the isolates of the Coagulase-negative *Staphylococcus* had the lowest ratio, 33 (8%), While the results showed that *E.coli* was the most prevalent among the 186 gram-negative isolates with a percentage of 128 (31.6%), followed by *Klebsiella pneumoniae* with 37 (8.97%), followed by *Pseudomonas aeruginosa* bacteria with 14 (3.39%), followed by *Proteus mirabilis* by 7 (1.96%), as shown in Table (1).

Table 1: Distribution of bacteria isolated from semen

Bacterial isolation	Number	Percentage (%)	P-value
Gram positive bacteria			
Staphylococcus aureus	107	(25.97)	**P < 0.05**
Coagulase negative Staphylococci	33	(8.00)	
Streptococcus agalactiae	78	(18.93)	
Gram negative bacteria			
E.coli	128	(31.06)	**P < 0.05**
Proteus mirabilis	7	(1.69)	
Klebsiella pneumoniae	37	(8.98)	
Pseudomonus aeruginosa	14	(3.39)	
MIX Growth	8	(1.94)	
Toal	412	%100	

The results of the sensitivity assay for Gram-positive bacteria isolates showed that *Staphylococcus aureus* bacteria were highly sensitive to Amikacin (91.1%), followed by Levofloxacin (90.7%), Meropenem, and Doxycycline (88.8%), followed by Rifadin (65.4). %), then the antibiotic Ciprofloxacin (57%) and the antibiotic Garamycin (38.3%). While *Staphylococcus aureus* bacteria were highly resistant to the antibiotics Ampiclox, Cefalexin, Amoxicillin, and Azithromycin (100%), followed by the antibiotics Cephalothin and Trimethoprim (99%), then the antibiotics Nalidellic acid (98.1%) and the antibiotics Clarithromycin and Cefixime Resistance by (95.3%). As for *Coagulase-negative Staphylococcus* bacteria, they were (100%) sensitive to the antibiotic Levofloxacin, followed by Meropenum (93.9%), as well as sensitive (90.9%) to Amikacin, followed by Doxycycline (87.9%), followed by the antibiotics Ciprofloxacin and Rifadin(78.8%) and(72.7%) respectively. While *Coagulase-negative Staphylococcus* bacteria showed high resistance to the antibiotic Cefixime (96.9%), they were followed by the antibiotics Norfloxacin, Amoxicillin, and Trimethoprim (93.9%). While antibiotics had very high resistance rates to *Coagulase-negative Staphylococcus* bacteria, exceeding the sensitivity rates in table (2).

The results showed that *Streptococcus agalactiae* was sensitive to Levofloxacin (93.6%), followed by Meropenum (91%), Amikacin (88.5%), Doxycycline (83.3%), and Rifadin (70.5%), Ciprofloxacin (66.7%) and Garamycin (51.3%). *Streptococcus agalactiae* was highly resistant to Amoxicillin and Nalidellic acid (100%), followed by Ampiclox, Cephalothin, and Cefalexen (98.7%), and the rest of the antibiotics. The resistance ratios were also high, amounting to more than (50%) more than its sensitivity, as shown in Table (2).

Table 2: Antibiotic sensitivity assay for Gram-positive bacteria associated with semen

Antibiotics	***Staphylococcus aureus* NO.(%)**		***Coagulase negative Staphylococci* NO.(%)**		***Streptococcus agalactiae* NO.(%)**	
	S	*R*	*S*	*R*	*S*	*R*
Ciprofloxacin	61(57)	46(43)	26(78.8)	7(21.2)	52(66.7)	26(33.3)
Clarithromycin	5(4.7)	102(95.3)	7(21.2)	26(78.8)	6(7.7)	72(92.3)
Claforane	14(13.1)	93(86.9)	16(48.5)	17(51.5)	13(16.7)	65(83.3)
Rifadin	70(65.4)	37(34.6)	24(72.7)	9(27.3)	55(70.5)	23(29.5)
Garamycin	41(38.3)	66(61.7)	18(54.5)	15(45.5)	40(51.3)	38(48.7)
Meropenum	95(88.8)	12(11.2)	31(93.9)	2(6.1)	71(91)	7(9)
Amikacin	98(91.6)	9(8.4)	30(90.9)	3(9.1)	69(88.5)	9(11.5)
Levofloxacin	97(90.7)	10(9.3)	33(100)	0(0)	73(93.6)	5(6.4)
Ceftiraxone	11(10.3)	96(89.7)	11(33.3)	22(66.7)	8(10.3)	70(89.7)
Doxycycline	95(88.8)	12(11.2)	29(87.9)	4(12.1)	65(83.3)	13(16.7)
Norfloxacin	6(5.6)	101(94.4)	2(6.1)	31(93.9)	8(10.3)	70(89.7)
Cefixime	5(4.7)	102(95.3)	1(3)	32(97)	12(15.4)	66(84.6)
Ampiclox	0(0)	107(100)	6(18.2)	27(81.8)	1(1.3)	77(98.7)
Cephalothin	1(1)	106(99)	4(12.1)	29(87.9)	1(1.3)	77(98.7)
Cefalexen	0(0)	107(100)	5(15.2)	28(84.8)	1(1.3)	77(98.7)
Amoxicillin	0(0)	107(100)	2(6.1)	31(93.9)	0(0)	78(100)
Trimethoprim	1(1)	106(99)	2(6.1)	31(93.9)	1(1.3)	77(98.7)
Nalidellic acid	2(1.9)	105(98.1)	3(9.1)	30(90.9)	0(0)	78(100)
Azithromycin	0(0)	107(100)	7(21.2)	26(78.8)	3(3.8)	75(96.2)
Nitrofurontion	7(6.5)	100(93.5)	14(42.4)	19(57.6)	8(10.3)	70(89.7)

The sensitivity assay results for Gram-negative bacteria isolates showed that *E.coli* showed high sensitivity to Levofloxacin (93.7%), Amikacin (89.1%), and other antibiotics. Meropenem and Doxycycline by (85.2%), followed by the antibiotics Rifadin and Ciprofloxacin with percentages (75.8%) and (57%), respectively, while the other antibiotics had *E.coli* sensitivity rates of less than (10%) and high resistance. Very range between (90% - 100%) as in Table (3).

The results showed that *Klebsiella pneumoniae* bacteria are sensitive to the antibiotic Levofloxacin (94.6%), followed by the antibiotics Amikacin and Meropenum (89.2%), followed by the antibiotics Rifadin and Doxycycline (54.1%) and (73%), followed by Ciprofloxacin by (51.4%) and Garamycin (45.9%). The results showed that most *Klebsiella pneumoniae* bacteria were highly resistant to the rest of the other studied antibiotics (100%), as in Table (3).

The results of the study showed that *Pseudomonas aeruginosa* bacteria are sensitive to the antibiotic Levofloxacin with a percentage of (92.9%), followed by the antibiotics Meropenum and Doxycycline at (85.7%), followed by the antibiotics Ciprofloxacin and Amikacin by (78.6%), followed by Garamycin (50%) and the antibiotic Rifadin by (42.9%). The results showed that *Pseudomonas aeruginosa* bacteria were highly resistant to the rest of the antibiotics in the study, with rates ranging between (11% - 100%) as in Table (3).

The results also showed that *Proteus mirabilis* bacteria are susceptible to the antibiotic Levofloxacin (100%), followed by the antibiotic Amikacin (85.7%), followed by the antibiotic Rifadin and Meropenum (71.4%), followed by Ciprofloxacin and Doxycycline by (57.1%). For the rest of the other antibiotics, the bacteria *Proteus mirabilis* was highly resistant to their direction (100%), as shown in Table (3).

Table 3. Antibiotic sensitivity assay for Gram-negative bacteria associated with semen

Antibiotics	***E.coli* NO. (%)**		***Klebsiella pneumoniae* NO.(%)**		***Pseudomonas aeruginosa* NO.(%)**		***Proteus mirabilis* NO.(%)**	
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*
Ciprofloxacin	73(57)	55(43)	19(51.4)	18(48.6)	11(78.6)	3(21.4)	4(57.1)	3(42.9)
Clarithromycin	10 (7.8)	118(92.2)	1(2.7)	36(97.3)	0(0)	14(100)	0(0)	7(100)
Claforane	20(15.6)	108(84.4)	3(8.1)	34(91.9)	2(14.3)	12(85.7)	1(14.3)	6(85.7)
Refadin	97(75.8)	31(24.2)	20(54.1)	17(45.9)	6(42.9)	8(57.1)	5(71.4)	2(28.6)
Garamycin	58(45.3)	70(54.7)	17(45.9)	20(54.1)	7(50)	7(50)	2(28.6)	5(71.4)
Meropenum	110(85.9)	18(14.1)	33(89.2)	4(10.8)	12(85.7)	2(14.3)	5(71.4)	2(28.6)
Amikacin	114(89.1)	14(10.9)	33(89.2)	4(10.8)	11(78.6)	3(21.4)	6(85.7)	1(14.3)
Levofloxacin	120(93.7)	8(6.3)	35(94.6)	2(5.4)	13(92.9)	1(7.1)	7(100)	0(0)
Ceftiraxone	17(13.3)	111(86.7)	4(10.8)	33(89.2)	3(21.4)	11(78.6)	2(28.6)	5(71.4)
Doxycycline	109(85.2)	19(14.8)	27(73)	10(27)	12(85.7)	2(14.3)	4(57.1)	3(42.9)
Norfloxacin	10(7.8)	118(92.2)	2(5.4)	35(94.6)	1(7.1)	13(92.9)	0(0)	7(100)
Cefixime	7(5.5)	121(94.5)	0(0)	37(100)	1(7.1)	13(92.9)	0(0)	7(100)
Ampiclox	4(3.1)	124(96.9)	1(2.7)	36(97.3)	0(0)	14(100)	0(0)	7(100)
Cephalothin	3(2.3)	125(97.7)	2(5.4)	35(94.6)	0(0)	14(100)	0(0)	7(100)
Cefalexen	2(1.6)	126(98.4)	0(0)	37(100)	0(0)	14(100)	0(0)	7(100)
Amoxicillin	2(1.6)	126(98.4)	0(0)	37(100)	0(0)	14(100)	0(0)	7(100)
Trimethoprim	0(0)	128(100)	0(0)	37(100)	0(0)	14(100)	0(0)	7(100)
Nalidellic acid	2(1.6)	126(98.4)	1(2.7)	36(97.3)	0(0)	14(100)	0(0)	7(100)
Azthromycin	4(3.1)	124(96.9)	0(0)	37(100)	0(0)	14(100)	0(0)	7(100)
Nitrofurontion	7(5.5)	121(94.5)	0(0)	37(100)	0(0)	14(100)	0(0)	7(100)

DISCUSSION

Bacterial infection may cause weakness in the reproductive system through a weakness in the process of sperm production, or it may cause inflammation and ulcers inside the tubes that carry sperm leading to temporary sterility[21]. Alternatively, it may lead to inflammation within the testicles or the seminiferous tubules are delicate and frequently twisted, causing weak sperm movement, lacking composition, low numbers, or preventing their exit[13]. In a similar study, it was observed that bacteria were found in 42 samples (52.5%). *E.coli* (13.7%) was the most common isolate, followed by *Klebsiella pneumonia* (11.2%), *Coagulase-negative Staphylococcus* (10%), *Staphylococcus aureus* (7.5%), *Streptococcus pyogenes* (6.2%) and *Pseudomonas aeruginosa* (3.7%)[22]. Another study consistent with our study showed that the most prevalent bacterial isolates in the semen were *Staphylococcus aureus* (77.8%) to be the most prevalent isolate[23].

In comparison, the percentage of other isolates was *E. coli* (11.1%) and *Citrobacter spp* (11.1%). The results of our study also agreed with the results of [15], where the most visible species were *Staphylococcus aureus*, *Coagulase-negative Staphylococcus*, *Streptococcus ssp*, *E.coli*, and sperm were in the samples containing *Staphylococcus aureus*, and *E. coli* are less numerous and less movable. Also, the results of our study were similar to those of [24, 18, 25, 26]. *E.coli* bacteria are among the most common microorganisms that infect people and cause temporary sterility if they are not detected and treated effectively because of their adhesion to the surface of the sperm, which affects its vitality and movement[16,27]. Another study indicated that the presence of *Staphylococcus aureus* bacteria in the semen causes a decrease in the number of sperms or a change in their external appearance[28].

Coagulase-negative Staphylococcus bacteria that naturally live on the surface of the urinary system or colonize the urinary system are opportunistic and cause suppuration of the semen during its descent from the genital tract, leading to a decrease in the efficiency or vitality of the sperm [2]. While the results of our study differ from the results of another study [29], the study included 30 positive samples for bacterial culture, the percentage of *Enterococcus faecalis* bacteria (30%) and was the most present in the semen, followed by *Coagulase-negative Staphylococcus* bacteria (23.33%), followed by *Staphylococcus aureus* (20%), *E.coli* (10%), while the percentage of bacteria *Klebsiella pneumonia* (6.66%), *Proteus sp* (6.66%), and *Citrobacter sp* (3.33%).In another study, the percentages differed from the percentages of our study as well, where the percentage of *Enterococcus spp* bacteria (30%), followed by *Klebsiella pneumonia* (24%), followed by *Staphylococcus aureus* and *Proteus spp* (18%). At the same time, *E.coli* was a little present [30]. Also, the results of our study differ from those of other studies[31, 19, 32, 16, 14, 7, 33]. This difference between the results of these studies and the results of our study is due to the different geographical regions from which the study samples were collected, as well as the types of bacterial isolates that may be more endemic in these areas than in our study area, and to a difference in the resistance of these bacterial isolates to antibiotics from one region to another geographical. Bacterial isolates in the semen affect the percentage of fructose sugar present in the semen plasma, which is a source of energy necessary for the vitality and motility of active sperm[9, 27]. that the presence of bacteria in the semen activates the work of white blood cells to confront them, releasing effective oxygen to defend the body against bacteria[8]. This type of oxygen causes significant damage to the sperm proteins, so they die or cause them to weaken in their movement; the inability to fertilize the egg and the presence of bacteria in the semen causes significant damage to the sperm in severe cases, causing spermatozoa disease [29].

CONCLUSION

This study indicated that many bacterial species were present in the semen of people with temporary infertility. These bacteria types are *Staphylococcus aureus*, *Coagulase-negative Staphylococcus*, *Streptococcus agalactiae*, *E.coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, the presence of these bacterial species in the semen cannot be ignored because of their negative impact on the number, density, shape, and movement of sperm, leading to the failure of pregnancy and infertility, so it must be dealt with. With it and treating it correctly, the results of this study indicated that the best antibiotics to treat these bacterial species were Levofloxacin and Meropenem, Doxycycline, Amikacin, and Rifadin, respectively.

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