In-Vitro Antibiotic Susceptibility Pattern of Pathogenic Bacterial Species Isolated from Semen of Infertile Men in Misurata, Libya

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Abstract

Bbackground: Since bacteria can agglutinate and immobilize spermatozoa, bacterial infections have been linked to infertility. According to this theory, there is a direct link between inflammation and male infertility. Objective: To study the microbiological profile of the semen specimens collected from adult married males visiting the infertility centers and investigate the antibiotic sensitivity pattern to these isolates. Material and method: Semen samples of 245 men attending Lamis IVF and Almajd Lab were evaluated with bacteriological culture. The specimens were processed using standard microbiological procedures for isolating and identifying the organism, followed by antibiotic susceptibility testing. The results were recorded and analysed. Results: The semen culture was negative in 40.80% and positive in 59.20% of the samples. From 145 samples, 77.90% Gram positive and 22% Gram negative. The most commonly isolated organism was Staphylococcus aureus (in 68.3% of the samples), followed by Escherichia coli (9.6%). This study revealed that Nitrofurantoin was active against most of the organisms isolated and can be used as drug to treat antibiotic-resistant bacteria for genitourinary tract infections; but most of the organisms were resistant against Tetracycline and Erythromycin. Conclusions: Bacterial infections can play an important role in male infertility and Nitrofurantoin and Imipenem can be potentially used for the treatment of antibiotic-resistant genital tract infections.

Keywords: bacteria, Male infertility, Antibiotic susceptibility.

Introduction:

A health problem that affects 10% of people worldwide is infertility. This phrase describes a couple's inability to become pregnant after a year of consistent, unprotected sexual contact. This occurs in 80–85% of undetermined couples after a year [1]. Infertility affects 13-15% 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].

Numerous things, such as malfunctioning accessory glands, oxidative stress, structural impediments in the seminal tube, and microbial infections that directly affect the quality of the semen, can cause inflammation [4]. Male reproductive function may be impacted by infectious disorders in a variety of ways, including by chronic infections and the resulting inflammation that can worsen spermatogenesis, impair sperm function, and/or clog the seminal duct. Immune-mediated infertility can be brought on by genital tract infections (GTI) and the development of antisperm antibodies in men. In conclusion, bacterial diseases of the genital tract have similar causal agents to those that cause urinary tract infections [5].

Numerous researches [6,7,8] have shown that 8–32% of instances of male infertility were related to infections and inflammations of the male genitourinary tract caused by pathogenic microorganisms like bacteria, viruses, fungi, and protozoa. Issues with infertility brought on by infections with these pathogenic agents include sperm destruction, 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].

However, there seems to be a connection between bacteriospermia and leukocytospermia in sub-fertile males. Male genitourinary system infections in the testis, epididymis, and prostate can harm spermatogenesis and reproductive potential. [2]. The purpose of this study was to determine the prevalence of microbial incidence in sperm and its

relationship to sperm quality and the effect of antibiotics on the isolates in infertile males.

Materials and Methods

Study design and subjects:

Adsorptive study design was utilized from the 1st of January 2020 until 30th of Sep. 2021. A total number of 245 male patients aged from 20-60 years' old who were sub-fertile or infertile were included in this study under the following criteria: has primary infertility one year or more, or secondary infertility two years or more. Both couples were not using any form of contraception. They had no other diseases affecting their fertility. Azoospermia obstructive or non-obstructive were excluded. They had no disease which affecting sperm parameters. 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.

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 [11].

Semen culture

After ejaculation, seminal fluid specimens were placed on the workbench for 20 minutes to dissolve before being delivered. Chocolate agar, Blood agar, MacConkey agar, and mannitol salt agar were among the solid media used to inoculate 0.01ml 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 by adding water to a gas generator envelope at the same temperature [12].

API 20E kits (for identification of Enterobacteriaceae)

The incubation box (tray and lid) was prepared by distributing 5 ml of distilled water into the wells of the tray to create a humid atmosphere. The strain reference was recorded on the elongated flap. The strip was removed from individual packaging and placed in the incubation box. The organism was cultivated onto nutrient agar 18-24 hours at 37°C. About four to five colonies were transferred to the API 20E. The micro-tubes were filled with the inoculated API 20E medium by using a pipette. The cupules of ADH, LDC, ODC and URE were filled with mineral oil to ensure anaerobic condition. Fill the cupules of CIT, VP and GEL completely by suspension. Close the incubation box and incubate at 37oC for 18-24 hours. Read the strip after 18-24 hours at 37oC.

Antibiotic susceptibility testing

The plates were incubated for 24 hours at 37°C, and the diameter of the inhibition zone around each of the antibiotic disks was measured and compared with (CLSI, 2021). Multidrug resistance was reported as a single isolate resistant (intermediate or complete), and the antibiotics used are: Sulfaprim 50 µg SXT, Cefoxitin 10 µg CEX, Ceftriaxone 30 µg CRO, Imipenem 10 µg IPM, Ampicillin 10 µg AM, Amoxicillin + clavulanic acid 25/10 µg AMC, Nalidixic acid 30 µg NA, Nitrofurantoin 50 µg F, Norfloxacine 5 µg NOR, Cefuroxime 30 µg CXM, Tetracycline 30 µg TE, Ciprofloxacin 5 µg CIP, Erythromycin 30 µg E, Fusidic acid 10 µg FA

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.

Results and discussion

All of the men were typically in good health, free of sex-related illnesses or chronic urogenital issues. None of them was receiving antibiotic treatment when the samples were collected. The ultimate number of patients enrolled in the trial was 245 as a result of a total of 13 men being excluded for one or more of the exclusion criteria. Out of the total 245 semen samples, 59.2% showed a significant bacterial growth, with the remaining 40.8% showing no growth (figure 1). These findings were consistent with those of Eini F., 2021, who noted that 60 (34.88%) of the 172 individuals who had been evaluated for infertility had a positive culture for harmful bacteria of various types. [5]. In the same line, Merino G.,1995 found that out of 183 patients, 119 (66%) had a positive culture [5].

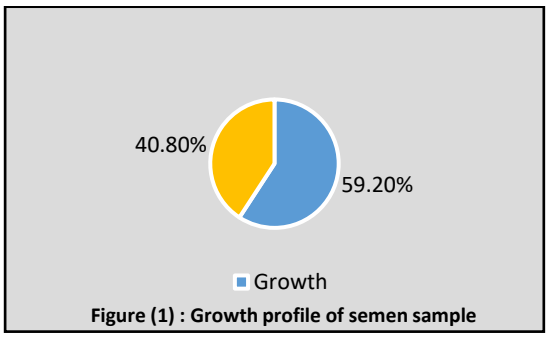

Figure (1) : Growth profile of semen sample

However, our finding was slightly higher than the research done in Kathmandu Medical College Public Limited, Kathmandu Nepal which showed that only 17.8% of samples showed a significant bacterial growth. Moreover, Berjis K, 2018 found that the percentage of positive cultures was about 21%.[1] The differences between these results could be attributed to a variation in the semen properties, the study design, the geographical

location, and/or epidemiological differences in the etiological agents [11]

From the isolated bacteria, Gram +ve bacteria were more prevalent than Gram -ve bacteria in which there were 32 isolates were from Gram -ve bacteria and 113 isolates were from Gram +ve bacterial organism (figure 2). The observation in this study showed that Gram +ve bacteria were predominant compared with Gram -ve bacteria which was in line with other studies conducted in India who found that about 67% of the isolates were Gram +ve cocci and 33.3% isolates were Gram -ve bacilli [22]. Similarly, the treatment center Qom-Iran results showed that 90.5% of isolates were Gram +ve cocci and 9.5% were Gram –ve[1]. However, results obtained in this study was contradictory to other reports, such as a study conducted in Kathmandu Nepal who mentioned that 39 isolates were from Gram -ve and 23 isolates were from Gram +ve bacterial organism. This difference might be due to the variation across geographical boundaries [3].

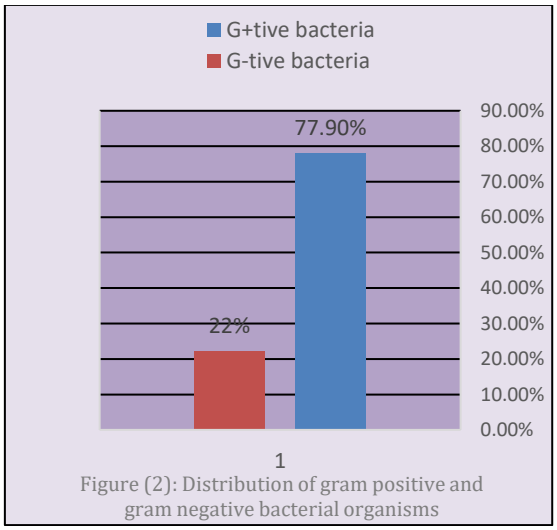

Figure (2): Distribution of gram positive and gram negative bacterial organisms

Spermioculture for the patient's semen showed that the most abundant bacterial genera were S. aureus; 99 isolates (68.3 %), followed by Uropathogenic *E. coli* (UPEC), 15 isolates (10.3%), *Neisseria gonorrhea*, 13 isolates (9.0%), *Staphylococcus saprophyticus* and *Staphylococcus epidermides* in 6 isolates each (4.1%), *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Klebsiella pneumoniae* in two isolates (1.4%) table (1). The P. value was 0.083 which means there is no statistically significant differences between the bacterial isolates.

Table (1): Species wise distribution of semen pathogens

Type	Pathogens	No.	Percen.
G+ve	*Staphylococcus aureus*	99	68.3%
	Staphylococcus saprophyticus	6	4.1%
	Staphylococcus epidermides	6	4.1%
	Streptococcus pyogenes	2	1.4%
G-ve	*Neisseria gonorrhea*	13	9.0%
	Uropathogenic Escherichia coli	15	10.3%
	Pseudomonas aeruginosa	2	1.4%
	Klebsiella pneumoniae	2	1.4%

In the same line a study conducted in Iran showed that 61.9% of semen samples was *S. aureus*. However, there has been constant data regarding to the semen bacterial content in which there is a great variability in different studies on the incidence of bacterial isolation in the semen of fertile and infertile men. Most of the collected data from previous studies named the same causative agents of urogenital tract infections, such as UPEC, *S. aureus*, *N. gonorrhea*, etc. with some differences the percentage of each one. Some authors have suggested that other bacteria, responsible for the colonization and contamination of the male urogenital tract, rather than infection, could also contribute to the decrease in sperm quality [1, 4].

The effect of antibiotics on the isolated bacteria was assessed by using disc diffusion method. The observed drug susceptibility pattern of Gram +ve isolates showed a high level of resistance to tetracycline (53.1%) and Nalidixic acid (38%). There was also an intermediate level of resistance (60%–80%) to SXT (73.50 %), CEX (69.9%), and E (64.6%). However, *Staphylococcus* isolates which considered as the predominant bacteria were highly sensitive to CIP and CXM. These results in constant with some of the studies that showed that most of the Gram +ve bacteria were found to be sensitive to [22, 23, 24].

Antibiotic	*S. aureus* (N= 99)			*S. saprophyticus* (N= 6)			*S. pygenous* (N= 2)			*S. epidermides* (N=6)		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
SXT	**28**	**71**	**0**	**0**	**6**	**0**	**0**	**2**	**0**	**2**	**4**	**0**
CEX	**30**	**69**	**0**	**2**	**4**	**0**	**0**	**2**	**0**	**2**	**4**	**0**
CRO	**56**	**37**	**6**	**4**	**2**	**0**	**2**	**0**	**0**	**4**	**2**	**0**
IPM	**60**	**27**	**12**	**6**	**0**	**0**	**2**	**0**	**0**	**5**	**1**	**0**
AM	**50**	**30**	**19**	**6**	**0**	**0**	**2**	**0**	**0**	**3**	**1**	**2**
AMC	**90**	**0**	**9**	**6**	**0**	**0**	**2**	**0**	**0**	**4**	**0**	**2**
NA	**40**	**19**	**40**	**6**	**0**	**0**	**2**	**0**	**0**	**3**	**0**	**3**
F	**91**	**0**	**8**	**6**	**0**	**0**	**2**	**0**	**0**	**6**	**0**	**0**
NOR	**88**	**11**	**0**	**6**	**0**	**0**	**2**	**0**	**0**	**4**	**2**	**0**
CXM	**99**	**0**	**0**	**6**	**0**	**0**	**2**	**0**	**0**	**6**	**0**	**0**
TE	**0**	**50**	**49**	**0**	**0**	**6**	**0**	**0**	**2**	**0**	**3**	**3**
CIP	**99**	**0**	**0**	**6**	**0**	**0**	**2**	**0**	**0**	**6**	**0**	**0**
E	**38**	**61**	**0**	**0**	**6**	**0**	**0**	**2**	**0**	**2**	**4**	**0**
FA	**50**	**49**	**0**	**4**	**2**	**0**	**1**	**1**	**0**	**3**	**3**	**0**

Table (2): The sensitivity of gram-positive bacteria to antibiotics

Table (3): The sensitivity of gram-negative bacteria to antibiotics.

Antibiotic	*UPEC* (N=15)			*K. pneumonia* (N= 2)			*P. aeruginosa* (N= 2)			*N. gonorrhea* (N=13)		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
SXT	**0**	**3**	**12**	—	—	—	—	—	—	**9**	**0**	**4**
CEX	**0**	**0**	**15**	**1**	**1**	**0**	**2**	**0**	**0**	**10**	**0**	**3**
CRO	**6**	**0**	**9**	**1**	**1**	**0**	**2**	**0**	**0**	**9**	**4**	**0**
IPM	**15**	**0**	**0**	—	—	—	—	—	—	**13**	**0**	**0**
AM	**0**	**0**	**15**	—	—	—	—	—	—	**0**	**6**	**7**
AMC	**10**	**0**	**5**	**1**	**0**	**1**	**2**	**0**	**0**	**5**	**8**	**0**
NA	**9**	**6**	**0**	—	—	—	—	—	—	**0**	**13**	**0**
F	**11**	**4**	**0**	**1**	**1**	**0**	**2**	**0**	**0**	**10**	**0**	**3**
NOR	—	—	—	—	—	—	—	—	—	**13**	**0**	**0**
TE	**0**	**0**	**15**	—	—	—	—	—	—	—	—	—
CIP	**0**	**15**	**0**	—	—	—	—	—	—	—	—	—
E	**0**	**0**	**15**	**2**	**0**	**0**	**2**	**0**	**0**	**10**	**3**	**0**
FA	—	—	—	—	—	—	—	—	—	**7**	**6**	**0**

Moreover, the antibiotic susceptibility test for Gram -ve bacteria revealed a high level of resistance AM (77.7%) while there was a variation between the other Gram -ve bacteria species in which *K. pneumoniae* and *P. aeruginosa* were sensitive to most of the used antibiotics. *N. gonorrhoea* showed a high sensitivity to most of the used antibiotics as shown in table 4 except for AM and NA while UPEC displayed a moderate effect for NA and CIP. Previous studies showed that most of the Gram -ve were found to be sensitive to IPM and F. Similar results were reported by other authors. [11, 22]

The UPEC and *Staphylococcus* spp. displayed a big variability in which the highest degree of resistance among the 15 antibacterial drugs was observed in UPEC which was resistant to 100% for AM, TE, CEX and E. TE was resisted 53.1% by *Staphylococcus* spp. The only antibiotic that showed 100% sensitivity to UPEC is IPM. There has been a large number of studies on the effects of antibiotic therapy on sperm quality and the pregnancy rate but there is no constant data in which some authors have obtained an improvement in semen quality and/or fertility in men with infected semen after antibiotic therapy, others have failed to corroborate these findings.

Study limitations: due to the lack of time, lack of budget, and corona quarantine situation, we could not study other semen parameters that help to detect the sperm quality.

Conclusion: The results of our study showed that bacterial semen infection could play a significant role in male infertility. However, the mechanism by which it affects fertility is complex and multifactorial. Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process. Two third of the samples were colonized with at least one

type of bacteria at concentrations high enough to have an impact on sperm quality. The predominant bacteria present in samples included *Staphylococcus aureus* and Uropathogenic *Escherichia coli*. Most of the Gram +ve cocci were sensitive to CIP, CXM and F while most of the Gram -ve bacilli were sensitive to F and IPM.

Recommendations: The collected results stress the importance of proper microbiological studies and specific antibiotic therapy in individuals suspected of having genital tract colonization, the semen microbial culture must be performed routinely with each patient expected to have a semen infection, 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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دراسة نمط الحساسية للمضادات الحيوية للأنواع البكتيرية الممرضة المعزولة من عينات المني للرجال المصابين بالعقم في مدينة مصراتة، ليبيا.

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الملخص

هدفت هذه الدراسة لعزل وتعريف البكتيريا الموجودة في عينات المني للرجال المصابون بالعقم ودراسة مدى قدرتها على مقاومة المضادات الحيوية، حيث تم فحص 245 عينة مني لرجال متزوجون مصابون بالعقم من مركز علاج العقم في مصحة لميس ومختبر المجد، حيث أظهرت النتائج بأن 40.8% من العينات لم تظهر أي نمو بينما أظهرت نموا إيجابيا في 59.2%، ومن إجمالي 145 عينة إيجابية النمو، كانت 77.9% منها بكتيريا موجبة جرام و22.1% سالبة جرام. وكانت بكتيريا *Staphylococcus aureus* هي السائدة في 68.3% من العينات تليها *E. coli* بنسبة 9.6%. وأظهر مضاد Nitrofurantoin فعالية ضد معظم البكتيريا المعزولة بينما كانت معظم البكتيريا مقاومة لمضادي Tetracyclineو Erythromycin .

الكلمات المفتاحية: عقم الرجال – المضادات الحيوية – عينات المني.