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ORIGINAL ARTICLE

Study of Bacteriospermia Isolated from Male Infertility in Al-Najaf City, Iraq

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ABSTRACT

Background: Bacteriospermia is the presence of bacteria in seminal fluid samples. Bacteriospermia can affect sperm function, leading to an abnormal fertility process. It is a major health condition that has a detrimental impact on male fertility and is usually caused by acute or persistent bacterial infections. It has been discovered that about 15% of infertile men and women are infertile. Bacterial infections can damage the prostate, epididymis, testis, and urethra, among other parts of the male genitourinary system. *Escherichia coli, Staphylococci, Streptococci*, and *Enterococcus faecalis* are the most commonly isolated pathogenic bacteria. On the other hand, the male urinary system is not fully sterile, as some bacteria, such as Staphylococcus epidermidis, have been found in otherwise healthy reproductive men. The effect of various bacteria on sperm quality is unknown at this time. The ever-increasing number of infertile couples has piqued scientific curiosity in the effects of bacteriospermia on male reproductive potential during the last few decades. To confirm the link between bacteriospermia and seminal characteristics, including motility and vitality, researchers looked into a variety of pathophysiologic pathways. Both direct bacterial interaction and immunological competent cell participation impact spermatogenesis, degrade semen function, and impede the urogenital canal.

Objectives: To explore the association between bacterial species found in guys' lower genital tracts and the quality of their sperm and male infertility.

Methods: The study included the amassment and examination of (175) samples of semen samples from infertile people for the period from 14/2/2021 to 17/5/2021, whose ages ranged between (19–40) years, isolation and identification of some bacteria by the automated VITEK-2 compact system utilizing Gram-negative identification cards (GN-ID) of bacteriosperm, the antibiotic sensitivity test was studied on bacteria isolates by the disk diffusion technique on Muller Hinton Agar (MHA).

Results: The highest bacterial infection rate among infected patients was *Staphylococcus haemolyticus*, which amounted to 26 isolates, followed by *E. coli* with 23 isolates, and 20 followed by *Staphylococcus epidermidsi* and *Staphylococcus saprophyticus* two isolates and

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Pseudomonas aeruginosa with one isolate for Show that there are consequential differences p < 0.05. the antibiotic susceptibility test. E. coli showed resistance to eight antibiotics, which are: However, most E. coli isolates are resistant to the antibiotics cefalexin, gentamicin, tetracycline, and levofloxacin. As for the sensitivity of *S. haemolyticus* to the antibiotics cefalexin, gentamicin, ceftriaxone, imipenem, amikacin, nitrofurantoin, Tetracycline, and Levofloxacin, it was resistant to five antibiotics: cefalexin, tetracycline, ceftriaxone, levofloxacin, and amikacin. While *S.epidermidis* bacteria were the most isolates resistant to cefalexin, tetracycline, But bacteria *S. pyogenes* was the most resistant to cefalexin as for *S. saprophyticus*, it was resistant to tetracycline.

Finally, *P. aeruginosa* was resistant to four antibiotics: Cefalexin, Tetracycline, Gentamicin, and amikacin.

Conclusion: Human sperm is high in nutrient-rich components, making it ideal for bacterial development. Normal skin flora, as well as harmful microorganisms, might contaminate it. Bacteria in sperm have been shown to affect sperm quality, according to research. Clinicians administer numerous antibiotics to treat infections of the urogenital tract and leukocytospermia. However, studies show that drugs used to treat infections negatively affect sperm parameters and male reproductive potential. Isolated strains from male infertility showed a significant proportion of antibiotic resistance, according to the study. Successful seminal fluid cultured were interpreted with caution, taking into account increased colony counts of unique isolation in the semen and elevated colony counts of multiple strains in the seminal fluid, the administration of antibiotics. As a result, the presence of seminal bacteria is a typical misinterpretation of genital tract infection.

INTRODUCTION

Bacteriospermia is bacteria in seminal fluid samples that can affect sperm function, resulting in an abnormal fertility process.[1] Reduced sperm motility, changed acrosome reaction, modified morphology, production of reactive oxygen species contributing to higher DNA fragmentation index, creation of anti-sperm monoclonal antibody as a result of a breach in the blood–testes barrier, and genital tract obstruction caused by inflammation and fibrosis.[2]

Bacterial presence in sperm has been demonstrated to alter sperm quality in various ways, including disrupting spermatogenesis, blocking the seminal tube, and compromising spermatozoa function. According to several studies,[3] starting motile sperm clumping reduces the ability of a crosome reaction, causing cell morphological changes, and produces reactive oxygen species created by the inflammatory response against bacterial infection.[4] Some bacteria produce a protein that has a direct effect on sperm motility. According to Prabha *et al*. (2009)[5] Sperm motility and viability are directly affected by a protein produced by *Staphylococcus aureus* termed Sperm Immobilization Factor (SIF). This bacteria can also be found in both men's and women's vaginal tracts. Bacteria can also cause spermatozoa to apoptosis by increasing the externalization of phosphatidylserine (PS) (a lipid present in the sperm membrane). PS externalization is regarded as the first step in sperm apoptosis, according to some research.

Villegas *et al.* (2005)[6] say that apoptosis may produce alterations in spermatozoa motility, vitality, and DNA integrity in male accessory gland infections without the need for exogenous reactive oxygen species (ROS) (MAGI). Infertility, miscarriages, and congenital disabilities can all be caused by DNA fragmentation produced by bacterial infection of sperm. Bacterial infections trigger the early appearance of histones. Reduced histone levels have been associated with lower fertility in mammals.

Antibiotics' deleterious effects on spermatogenesis and sperm function and their effects on male fertility have been reported across the animal kingdom. Antibiotics' effect on fertility and the implications for infertility treatment may be more complex than previously thought.

MATERIALS AND METHODS

The study included the amassment and examination of (175) semen samples from infertile people from 14/2/2021 to 17/5/2021, whose ages ranged between (19–40) years. Each sample was collected by the same patient Container that is sterile. Subjects were given instructions on collecting specimens and submitting them to the labs in one hour. They were instructed to urinate first, then wash their hands and penis with soap, wash, and finally masturbate and ejaculate in a sterile container. The sperm was collected. For at least 3 days, avoid sexual activity. Nutrient, MacConky, Mannitol salt, and Thayer Martin, eosin methylene blue, blood, kligler, peptone water, MR-VP, citrate, and chocolate agar were used to cultivate sperm. The media was incubated at 37°C for 24 hours. This media is used for *S. pyogenes, S. aureus, S. epidermidis, S. aureus, Escherichia coli, Proteus mirabilis, Proteus* common, *Klebseilla pneumoniae, Pseudomonas aeuroginosa, Neisseria gonorrhoeae.* Also, to confirm the culture results, we used microbial identification BIOMÉRIEUX VITEK® 2 SYSTEM

Gram stain to detect infectious bacteria, which were then cultured on a medium. According to Akiyama and Savin, (2010), microorganisms are identified and described and Macfadden, 2000 bacteriosperm antibiotic sensitivity was studied using the disk diffusion technique on Muller Hinton Agar (MHA), the disc manufacturer's specified inhibition zone criteria, and based on Barry's technique (1976).[7] Antibiotic discs were chosen as per the National Committee for Clinical Laboratory Standards' recommendations (NCCLS, 2002).

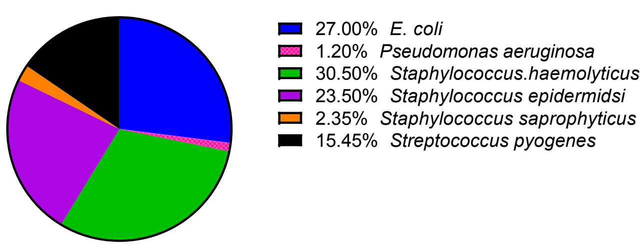
RESULTS AND DISCUSSION

Bacterial Study

Types of Bacteria

The results of isolating gram-positive and gram-negative bacterial species as shown in Table 1 and Figure 1 showed the number and percentage of bacterial isolates for each type of bacteria isolated. The highest bacterial infection rate among infected patients was *S. haemolyticus*, which amounted to (26) isolates, followed by *E. coli* with (23) isolates and (20) followed by *S. epidermidsi* and *S. saprophyticus* (2) isolates and Pseudomonas aeruginosa with 1 isolate for Show that there are consequential differences $p<0.05$.

Infection of the reproductive tract organs by bacteria can reduce sperm production or induce scarring and occlusion of the tubules that transport sperm, resulting in infertility by clogging the seminiferous tubules, which are small testicular tubules where sperm are created, infection of the testis can limit the production of sperm. Freshly conceived sperm are temporarily held in the epididymis, a testicular organ with coiled sperm ducts through which sperm undergo final maturation as they go through the sperm ducts. Infections of the epididymis can hamper sperm transport by interfering with the proper maturation of sperm. Because the prostate generates such a large amount of fluid in the ejaculate, infection



There are significant differences $p<0.05$.

Figure 1: The bacterial species isolated in the study.

Table 1: The bacterial species isolated in the study

Type of bacteria	*E. coli*	23
Gram-negative	*P. aeruginosa*	1
	S. haemolyticus	26
Gram positive	*S. epidermidsi*	20
	S. saprophyticus	2
	S. pyogenes	13
	Total	85

in the prostate can prevent fluid from reaching the prostate and decrease the volume of the ejaculate.[8]

Less frequently male infertility is due to non-sexually transmitted epididymo-orchitis, mostly caused by *Escherichia coli*. As a result, *E. coli* could be linked to the development of infertility.[9] Golshani *et al.*, (2006),[10] while studying the microbiology of semen samples of males who are unable to reproduce *E. coli* and Enterococci were discovered to be the most common bacteria that negatively affect sperm motility and morphology the impact of bacteriospermia on semen parameters. Schirren and Zander (1966)[11] have also discovered that when sperm and bacteria are mixed, *E. coli* negatively impacts sperm motility. *In vitro. E. coli* quickly attaches to human spermatozoa, leading in spermatozoa agglutination. A profound decline in the motility of spermatozoa is conspicuous over time due to rigorous alterations in sperm morphology. Morphological changes were found in all surface sperm structures, including the plasma membrane of the mid piece and neck and the acrosome's inner and outer acrosomal membranes, indicating that morphological deficiencies could be to blame for spermatozoa immobilization by *E. coli*. *E. coli* has virulence features that allow it to cling to sperm cells and colonize tissues of the male genital tract, resulting in asymptomatic male infertility.[12]

The findings also show that the bacteria that cause semen infection might come from the urinary tract or be spread through sexual contact. Unhealthy intercourse is the main thoroughfare for these germs to enter the reproductive tract.[13]

4-7: Antibiotic Sensitivity Test

The antibiotic susceptibility test results are shown in Table 2 and Figure 2. *E. coli* was resistant to eight antibiotics, including: Most *E. coli* isolates, however, are resistant to Cefalexin, Gentamicin, Tetracycline, and Levofloxacin. As for the sensitivity of *S. haemolyticus* to the antibiotics Cefalexin, Gentamicin, ceftriaxone, imipenem, amikacin, nitrofurantoin, Tetracycline, and Levofloxacin, it was resistant to five antibiotics: Cefalexin, Tetracycline, ceftriaxone, Levofloxacin, and amikacin. While *S. epidermidis* bacteria were the most isolates resistant to cefalexin, tetracycline, But bacteria *S. pyogenes* was the most resistant to cefalexin. As for *S. saprophyticus*, it was resistant to tetracycline.

Finally, *P. aeruginosa* was resistant to four antibiotics: Cefalexin, Tetracycline, Gentamicin, and amikacin. Antibiotic resistance among bacteria has been a big concern and ecumenical quandary, particularly among hospital bacterial infections, which has resulted in several risks and antimicrobial therapy benefits.[14] All of the test organisms used in this study were resistant to a variety of antibiotics. The fact that the bacterium replicates against antibiotics suggests that plasmids may play a role in resistance. Because plasmid-borne resistance is common in many bacteria, the presence of many plasmids may promote the high resistance profile against a variety of antibiotics.

These results are in agreement with many studies conducted by researchers including, researcher,[15] *S. faecalis* (31.4%), E. coli

Table 2: Antibiotic sensitivity test of bacteria

	Bacteria test																	
Antibiotics	*E. coli*			*S.haemolyticus*			*S.epidermidis*			*S. pyogenes*			*S. aprophyticus*			*P.aeruginosa*		
Sensitivity	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Cefalexin	2	9	10	3	5	18	10	4	8	0	4	9	1	0	1	0	0	1
Gentamicin	4	10	10	7	13	6	10	4	4	9	5	0	0	1	1	0	0	1
Tetracycline	5	8	8	6	6	14	7	8	8	6	2	4	0	0	2	0	0	1
Ceftriaxone	8	8	6	2	9	16	8	10	4	6	3	2	1	0	1	1	0	0
Imipenem	18	2	3	20	2	4	20	2	0	9	1	1	2	0	0	1	0	0
Amikacin	3	13	5	5	14	8	12	7	3	2	8	2	0	1	1	0	0	1
Levofloxacin	4	10	8	8	9	9	14	5	3	8	2	2	1	0	1	0	1	0
Nitrofurantoin	8	11	2	15	10	2	13	7	2	8	4	0	0	1	1	0	1	0

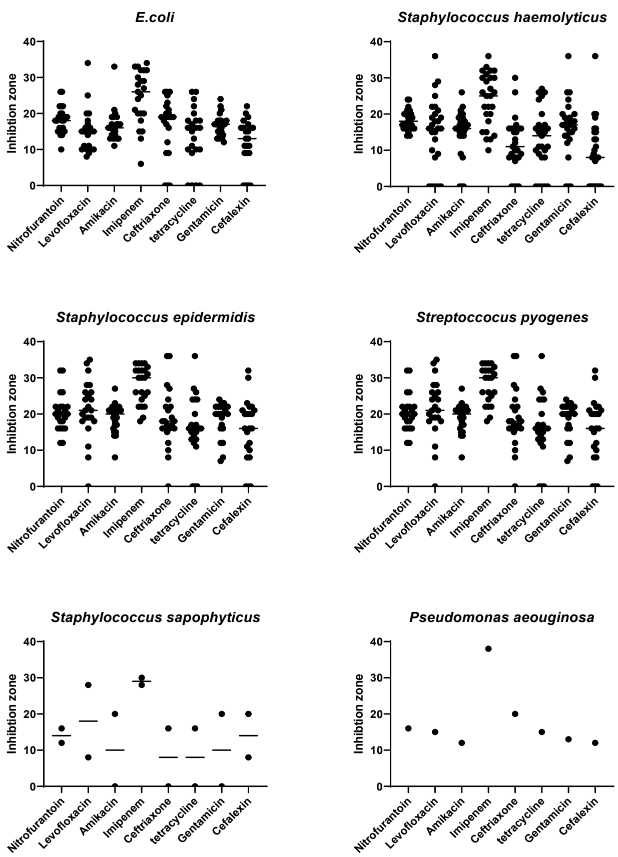


Figure 2: Antibiotic sensitivity test of bacteria

(17.1%), and coagulase-negative Staphylococci (32.9%) were discovered to have considerable bacterial amplification, and the organisms isolated were *S. faecalis* (31.4%), *E. coli* (17.1%), and coagulase-negative Staphylococci (32.9%). (14.3 percent). The most common isolate was *S. aureus* (37.1 percent).

Rehewy *et al.* (1979)[16] obtained 73 percent positive bacterial cultures. The most mundane aerobic organisms grown are Corynebacterium, *S. aureus, S. epidermidis, E. coli, P. mirabilis, K. pneumoniae, and Mycoplasma.* Ekhaise *et al.* (2011)[17] found that *S. aureus* 7 (77.8 percent) was the most prevalent isolate. However, it has been suggested that bacteria in semen can disrupt fertility in various ways, including causing sperm damage, affecting motility, and changing the chemical composition of semen. According to Karpuz *et al.* (2007),[18] sperm infection can cause persistent UTI by functioning as a reservoir for infection. Mogra and his colleagues (1981).[15] In many cases, opportunistic bacteria cause classic and subclinical urogenital infections, such as the epididymis and prostatitis. The presence of microorganisms in the ejaculate has been linked to specific potential pathophysiological pathways that lead to the development of infertility. Semen microbial infection is a primary cause of male infertility, according to Rehewy *et al.* (1979),[16] Gomez *et al.* (1979).[19] The viability and structural integrity of sperm is the hallmark of mobility. Stephen *et al*., (1989)[20] The negative effect of these microorganisms on the reproductive capacity of spermatozoa were revealed in cases of *E. coli* and *S. aureus,* among other microorganisms. Sanocka - Macleiewska *et al*., (2005)[21] Presence of *E. coli* in semen decreases sperm movement. Auroux *et al*., (1991)[22] Bacteria influence sperm movement through adhesion, agglutination, and lysis factors. It is known that there is a very significant negative effect of *E. coli*, *Streptococci,* and coagulase-negative *Staphylococci* on sperm movement, morphology, and viability (Esfandiari *et al*., 2002).[23]

Antibiotic resistance is frequently plasmid-borne, as has been widely documented. Antibiotic resistance was highest in *Proteus vulgaris*, with a value of six out of eleven antibiotics (54.5 percent). Proteus vulgaris has been found to be resistant to Cotrimoxazole and Nitrofurantoin. The high antibiotic resistance of *P. vulgaris* may denote the resistance levels among the Enterobacteriaceae because indiscriminate ingestion of antibiotics provides selective pressure, leading to a higher (Carattoli, 2008)[24] prevalence resistant bacteria, according to Lyskova *et al*., (2007).[25] These species are potential infection sources and possible carriers of resistance genes that might be passed on to other bacterial pathogens. The large calibers of ß-lactamase produced and the isolates' multi-drug resistance are indicators of an increase in the resistance threat (Thyagarajan *et al*., 2003)[26] reported by many studies. The presence of plasmids is crucial for resistance in Staphylococcus aureus, which is the second most resistant bacterium in this study. Plasmids are thought to play a critical role in mediating and transmitting antibacterial drug resistance in the *Staphylococcus* population. They can be vectors for resistance genes, or they can be found in discrete transposable elements of DNA known as transposons, which are mobile and can hop from one DNA molecule to the next. This can hasten the spread of antibiotic resistance in a staphylococcus community and explain why multi-resistant forms evolve. *P. aeruginosa*, on the other hand, displayed a high level of multiple resistance. This is in line with the findings of (Keskin *et al*., 2010),[27] who found that *P. aeruginosa* is typically resistant to a wide range of medications. *E. coli's* numerous resistances to amoxicillin, chloramphenicol, and tetracycline are consistent with the findings of (Chamot *et al*., 2003),[28] who revealed multiple resistances of the organism beta-lactam antibiotics. Amoxicillin resistance was found in five of the seven test organisms (71.4%), while Augmentin resistance was found in four of them (57.1%). Adeshina *et al.* (2012)[29] support the findings that amoxicillin and tetracycline have significant optical resistance, which could be related to their widespread and long-term usage and abuse in our society. With one organism each, Nalidixic, Chloramphenicol, and Cloxacillin were the least resistant (14.3 percent). With all the results accedes with researcher Bhatt *et al*. (2015),[30] *E. coli* was found susceptible to nitrofurantoin (76.9%), followed by levofloxacin (69.2%), ampicillin-sulbactam (57.6%), gentamycin (61.5%), and co-trimoxazole (50%). S. aureus was found 81.83% sensitive to nitrofurantoin, followed by levofloxacin (63.6%) and gentamycin (54.5%). Mogra *et al.* reported a maximum number of *Staphylococcus albus*, *S. faecalis,* and *E. coli* were sensitive to ampicillin, followed by trimethoprim-sulphamethoxazole (co-trimoxazole), nitrofurantoin, erythromycin, and chloramphenicol. Mogra *et al*., (1981)[15] bacterial resistance to ciprofloxacin, ofloxacin, cephalexin and gentamicin may be linked to the overuse and indiscriminate use of these medicines. The emergence of antibiotic-resistant microorganisms is influenced by insufficient antimicrobial therapy. Greetings, Marin (2000);[31] according to the findings of this investigation, bacterial pathogens were discovered in 17.8% of semen cultures. The organism *E. coli* was found to be the most frequent, followed by *S. aureus.* The age range of 31–40 years old was the most common for culture-positive cases. Nitrofurantoin appears to be the medicine of choice for empirical treatment, followed by levofloxacin and ampicillin sulbactam. The traditional bacterial pathogen screening in infertile males appears to be needed since it influences infertility in numerous ways.

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