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Bacterial Infection in Male Infertility in Al-Anbar Province West Of Iraq.

Dhuha A. Abbas¹, Abbas O. Al.janabi², Waleed N. ³

1- Bacteriology Department, Falluja Teaching Hospital ,AL-Anbar province.

2- Microbiology Department , College of Medicine, University of Anbar.

3-Surgery Department, College of Medicine , University of Anbar

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ABSTRACT

Background: The role of bacterial infections on male infertility has always been controversial due to the lack of decisive analytical tools for examining seminal fluid specimens. As a result, these infectious processes lead to deterioration of spermatogenesis, impairment of sperm function and/or obstruction of the seminal tract.

Aims of the study: To investigate the role of bacterial infections in male infertility in the province of Al - Anbar, western Iraq and their pattern of susceptibility to antibiotics in vitro.

Patients and methods: Semen specimen were collected and processed in accordance with standard microbiology techniques for routine culture and antibiotic susceptibility.

Results: Out of 80 semen samples, bacteriospermia was observed in 42(52.5%). *E. coli* took first rank of isolation (13.7%), followed by *Klebsiella pneumoniae* (11.2%), while Coagulase negative *Staphylococcus* was shown in (10%). Regarding antibiogram, *E. coli* was found to be susceptible to Meropenem (100%), followed by Amikacin(81.8%) and Levofloxacin (81.8%).

Conclusion: We can conclude from the study that is semen culture is an important diagnostic tool for all patients undergoing fertility investigations and bacteriospermia is an important cause of infertility, and for empirical treatment levofloxacin and meropenem seems to be drug of choice. The regular screening of bacterial pathogen in infertile man seems necessary because it affects infertility in several ways

INTRODUCTION

According, to, the, World Health Organization (WHO), the International Committee for Monitoring Assisted Reproductive Technology. Infertility is an illness of the reductive, system that is defined as the failure to achieve clinical pregnancy following a period of 12months or more of regular unprotected sexual intercourse (Kumar a n d S i n g h , 2015).

Acute and chronic infections and subsequent male reproductive inflammation may affect sperm function as well as the spermatogenic process causing qualitative and quantitative sperm changes (Moretti *et al.*, 2009). By any of these mechanisms, bacteriospermia affects the normal fertility process: Reduced motility of sperm, spermatogenesis deterioration, altered reaction of the acrosome, reactive oxygen species formation leading to a higher DNA fragmentation index, morphological alterations, anti-sperm antibodies formation because of breaches of the blood-testes barrier and blockage of the genital tract due to inflammation and fibrosis (Vilvanathan *et al.*, 2016). The bacteria responsible for semen contamination usually originate in patients' urinary tract or can be transmitted via sexual intercourse by the partner (Moretti *et al.*, 2009). It is difficult to distinguish active genitourinary tract infections from commensal microflora. Infections of the male genital tract are difficult to detect since they are often asymptomatic and often remain undiagnosed, except for specific symptoms (Kiessling *et al.*, 2008). However, the diagnosis of "silent" genital tract infections should receive consideration, given the growing number of patients seeking treatment with assisted reproduction for impaired fertility (intrauterine insemination, in vitro fertilization or intracytoplasmic sperm injection), as the infection may be related to asthenozoospermia (Villanueva-Diaz *et al.*, 1999). Infections are potential causes of male infertility and resistance to standard antibiotics, and poor conformity may impair the effectiveness of antibiotics in the resolution or restoration of complicated pyospermia (Pallett and Hand, 2010). The relationship between infertility and the presence of pathogenic microorganisms in the reproductive tract is widely established (Gdoura *et al.*, 2008).

MATERIALS AND METHODS

In this study, 80 semen specimens were collected from infertile patients for

routine cultivation and antibiotic sensitivity testing and processed in accordance with standard laboratory procedures. Semen was collected in clean dry, sterile and leak-proof container after 2-3 days of sexual abstinence in aseptic condition. The collected sample was evaluated with respect to its acceptability, proper labeling (patients name, age, date, time of collection and patient serial number). The semen specimen were cultured onto the blood agar and MacConkey agar plates using the semi-quantitative culture technique. By using standard calibrated loop (0.001 ml) of the uncentrifuged mixed sample was cultured on blood and MacConkey agar. Then these plates were incubated aerobically at 37°C for 24 hours.

Bacterial isolates were identified using standard microbiological techniques as outlined in Bergey's manual of systemic bacteriology which includes studying the character of the colony, staining reactions and biochemical testing (Geoge MD, David RB, 2001).

Kirby - Bauer disk diffusion method was used to test different isolates for antibiotic susceptibility according to CLSI recommendations (CLSI 2018). The discs of antibiotic used for susceptibility were from bioanalyses laboratories. Limited such as Meropenem (10µg), Levofloxacin (30µg), Trimethoprim (10µg), Azithromycin (15µg), Doxycycline (30µg), and Amoxicillin/clavulanic acid (30µg), Cefixime (5µg), Lomefloxacin (5µg), Ciprofloxacin (5µg), Ampicillin (10µg) and Amikacin (30µg) SPSS analyzed the data and presented it in tables and diagrams.

RESULTS

Sex and Hospitalization:

80 Samples of semen were received from infertile couple male partners. The mean age group of the study population was 29.88, the percentage of the primary infertility

was 56.3%, and secondary infertility was 43.8% (Fig. 1). Bacteriospermia was observed in 42 (52.5%) samples. *Escherichia coli* (13.7%) was the most common isolated organism followed by *Klebsiella pneumonia* (11.2%), Coagulase-negative *Staphylococcus*

(10%) , *Staphylococcus aureus* (7.5%) , *Streptococcus pyogenes* (6.2%) and *Pseudomonas aeruginosa* (3.7%) (Fig.2)

Antibiotic sensitivity testing using Kirby - Bauer disc method has been shown in table 1.

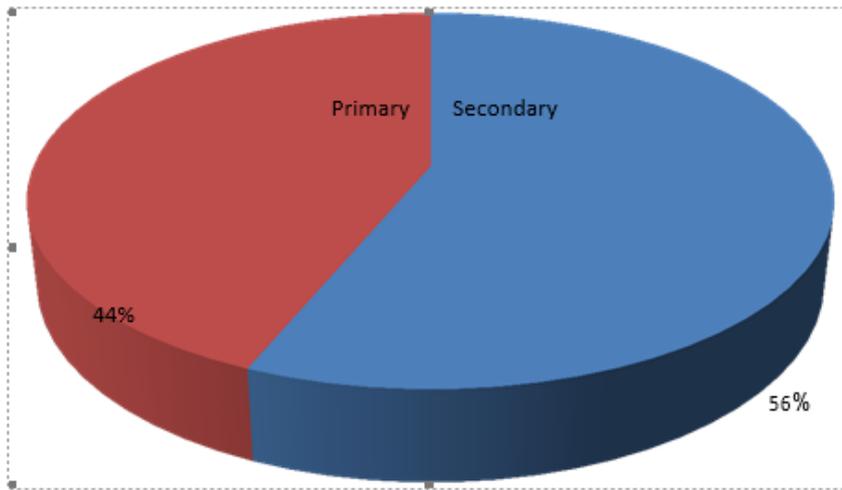


Fig.1 the percentage of primary and secondary infertility

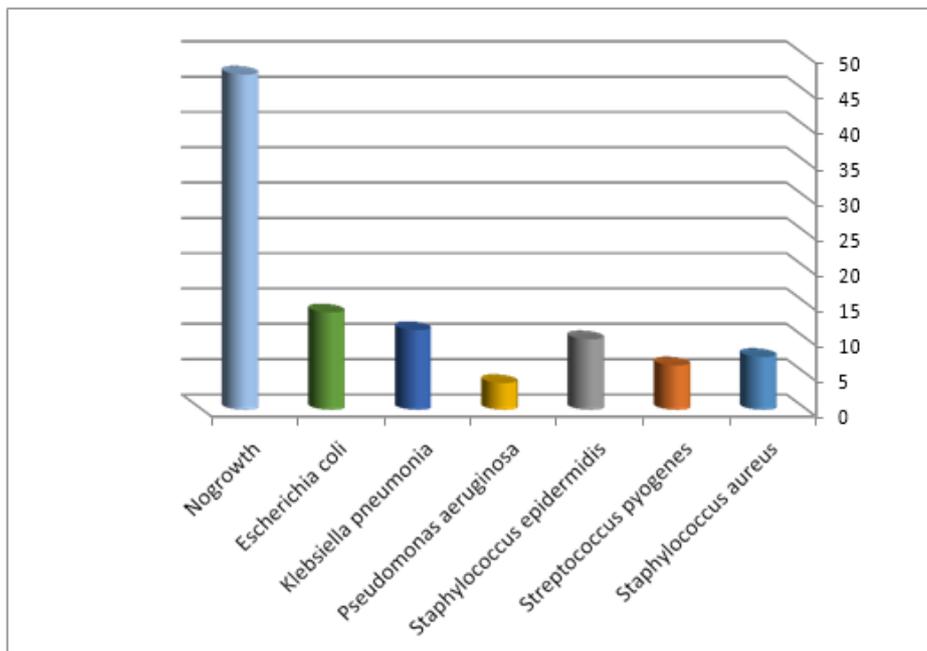


Fig.2 the percent of different isolated microorganism

Table 1: Antibiotic susceptibility profile of bacterial isolates.

Microorganism	AK	LEV	LOM	AZM	TMP	CIP	CFM	MEM	DO	AM	AMC
<i>Escherichia coli</i>	81.8	81.8	72.7	72.7	54.5	63.6	54.5	100	63.6	63.3	63.6
<i>Klebsiella pneumonia</i>	77.7	100	88.8	77.7	55.5	88.8	22.2	88.8	66.6	11.1	11.1
<i>Staphylococcus epidermidis</i>		87.5	75	87.5	62.5	62.5			62.5		
<i>Staphylococcus aureus</i>		83.3	0	33.3	33.3	16.6			33.3		
<i>Streptococcus pyogenes</i>		40		0						0	
<i>Pseudomonas aeruginosa</i>	66.6	100	33.3			66.6		100			

DISCUSSION

Many studies have examined the impact of bacteriospermia on infertility which remains controversial (Menkveld and Kruger 1998 , Omu *et al.* 1999 , Sanocka *et al.* 2004) . In this study, the prevalence of bacteriospermia was(52.5%).The similar prevalence rate was observed in a Study by (Golshani *et al.* 2006) .Higher prevalence rates of 65.7%, 66%, and 79% were shown by (Isaiah *et al.*, 2011 , Merino *et al.*, 1995,& Damirayakhian *et al.*, 2006) respectively.

Among the different isolated bacteria, *Escherichia coli* (13.7%) predominated in this study . In a few studies, however, *Staphylococcus aureus* and *S. epidermidis* have been found to be more commonly isolated (Rodin *et al.* , 2003,Isaiah *et al.*, 2011, Owolabi *et al.*, 2013 ,& Alekwe *et al.*, 2013) .

In the present study, *E. Coli* was found to be susceptible to Meropenem with (100%), followed Amikacin and Levofloxacin (81.8), Lomofloxacin (72.7%) , Azithromycin (72.7%) , Trimethoprim (54.5%) , Ciprofloxacin (63.6%) , Cefixime (54.5%) , Doxycycline (63.6%) , Ampicillin (63.3%) and Amoxicillin / clavulanic acid (63.6%) . *Klebsiella pneumonia* was found to be susceptible to Levofloxacin with percent (100%) , followed by Lomofloxacin, Ciprofloxacin and Meropenem the percentage was (88.8%) ,and for Amikacin and Azithromycin the

percentage was (77.7%) , Doxycycline was (66.6%) , Trimethoprim was (55.5%) , Cefixime (22.2%) and for Ampicillin and Amoxicillin / clavulanic acid the percentage was (11.1%). *Pseudomonas aeruginosa* was susceptible to Levofloxacin with percent (100%) , and for Ciprofloxacin and Amikacin the percent was (66.6%), *S.aureus* was susceptible to Levofloxacin with a percent (83.3%), while *S epidermidis* was (87.5%), Azithromycin the percent of susceptibility was (87.5%) for *S.epidermidis* and (62.5%) for *Trimethoprim* ,Doxycycline and Ciprofloxacin. Mogra *et al.* reported the highest number of strains of *Streptococcus faecalis* , *Staphylococcus* and *E. coli* which were sensitive to Ampicillin, Trimethoprim-sulphamethoxazole (co-trimoxazole), Nitrofurantoin Erythromycin and Chloramphenicol respectively(Mogra *et al.*, 1981) . and (Bhatt *et al.* 2015) was found that *E. coli* was susceptible to Nitrofurantoin (76.9%), followed by Levofloxacin (69.2%),Gentamycin (61.5%) , Ampicillin sulbactam (57.6%) and Co-trimoxazole (50%).*S. aureus* was found 81.83% sensitive to Nitrofurantoin ,Lavofloxacin (63.6%) and Gentamycin (54.5%) respectively.

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ARABIC SUMMARY

الأخماج البكتيرية في العقم عند الرجال في محافظة الأنبار _ غرب العراق

ضحى احمد عباس¹ , عباس عبيد الجنابي² , وليد نصار³

1-قسم البكتيريا مستشفى الفلوجة التعليمي العراق , الفلوجة

2-فرع الاحياء المجهرية كلية الطب جامعة الأنبار العراق , الرمادي

3-فرع الجراحة كلية الطب جامعة الأنبار العراق , الرمادي

جمعت ثمانون عينة سائل منوي من اشخاص يعانون من العقم للفترة من تشرين الثاني 2018 الى شباط 2019 زرعت جميع هذه العينات في الظروف الهوائية على وسط اكار الدم ووسط ماكونكي وحضنت جميع الاطباق لمدة 24 ساعه بدرجة 37 درجة مئوية .تم تشخيص العزلات الجرثومية بواسطة الاختبارات البايوكيميائية وصبغة كرام .وتم اجراء اختبار الحساسية للمضادات الحيوية للعزلات بطريقة كربي باور .اظهرت نتائج العزل وجود (42) عينة تحتوي على نمو بكتيري وكانت نسبة الاشريكية القولونية هي الاعلى بنسبة (% 13.7) ثم تليها بكتريا الكلبسيلا بنسبة (% 11.2) ثم المكورات العنقودية والزائفات الزنجارية . اما بالنسبة لاختبار الحساسية للمضادات الحيوية اظهرت النتائج ان عزلات الاشريكية القولونية والزائفات الزنجارية كانت حساسة لمضاد الميروبينيم بنسبة (% 100) اما عزلات الكلبسيلا كانت نسبة الحساسية (% 88.8) اما بالنسبة لمضاد الليفوفلوكساسين كانت نسبة حساسية الكلبسيلا والزائفات الزنجارية (% 100) لكليهما اما بكتريا الاشريكية القولونية كانت النسبة (. 81.8 %) .