



Study of Some Bacterial Isolates Associated with Leukocytospermia in Asthenospermic Patients in Hilla City, Iraq

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Abstract

One hundred asthenospermic seminal fluid specimens were collected from 100 infertile males who referred to Babylon maternity and children hospital-infertility center. It was found that 70 seminal fluid specimens had asthenospermia with leukocytospermia (First group). The rest 30 seminal fluid specimens had asthenospermia without leukocytospermia (Second group). Regarding semen cultures the results showed that 61(87.1%) of specimens of First group revealed positive bacterial culture, whereas 9(12.9%) specimens of First group showed no bacterial growth even after incubation of 48 hours. All semen specimens of Second group revealed negative bacterial culture. Gram positive bacteria constituted 44(62.9%) while gram negative bacteria constituted 26(37.1%) of isolates. Coagulase negative staphylococci (CoNS) represented by *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* were the common type of bacterial isolates 25(35.7%) followed by *Staphylococcus aureus* 19(27.2%), *Escherichia coli* 12(17.1%), *Enterobacter aerogenes* 8(11.4%), *Acinetobacter* spp 4(5.7%) and *Moraxella* spp 2(2.9%). The virulence factors of bacterial isolates were investigated. The results showed that all *S. aureus* isolates, 18(72%) isolates of CoNS and 5(41.7%) of *E. coli* isolates and 4(50%) of *E. aerogenes* isolates produce hemolysin. Colonization factor antigens (CFA/III) were detected in all isolates of *S. aureus*, CoNS, *E. coli*, *E. aerogenes*, *Acinetobacter* spp. and *Moraxella* spp. (CFA/I) were expressed in 10(52.6%) isolates of *S. aureus*, 8(32%) isolates of CoNS, 8(66.7%) isolates of *E. coli*, 6(75%) isolates of *E. aerogenes*, 2(50%) isolates of *Acinetobacter* spp. and 1(50%) isolate of *Moraxella* spp. Lipase produced by 15(78.9%) and 7(28%) isolates of *S. aureus* and CoNS isolates respectively, while 9(75%) isolates of *E. coli*, 7(87.5%) isolates of *E. aerogenes* and 1(50%) isolate of *Moraxella* spp. produce lipase. Only 7(36.8%) isolates of *S. aureus* and 5(41.7%) isolates of *E. coli* were found to be protease producers. The effects of some antibiotics on bacterial isolates were investigated. The results showed that, the bacterial isolates were highly susceptible to imipenem, meropenem and ciprofloxacin whereas exhibited moderate resistance to amikacin, gentamycin and norfloxacin. On the other hand bacterial isolates revealed high rate of resistance to amoxicillin, ceftizoxime, ceftazidime, cefamandole, cefepime, amoxicillin-clavulanic acid and tobramycin.

Keywords: Bacteriospermia, Asthenospermia, leukocytospermia, CoNS, colonization factor antigens.

Introduction

Male urogenital tract infection is one of the most important causes of male infertility, worldwide since genital tract infection and inflammation have been associated with 8-35% of male infertility cases¹. Bacteriospermia is defined as the presence of bacteria in seminal fluid samples². Bacteriospermia may play a major role in infertility^{3,4}. Male accessory sex glands infection is a major risk factor in infertility⁵. The significance of pathophysiology of bacteriospermia has been seriously discussed in recent years. Some possible pathomechanisms of the development of infertility linked with infection are considered: direct effect on sperm function (motility, morphology), deterioration of spermatogenesis, autoimmune processes induced by inflammation and dysfunction of accessory sex glands⁴. Hence, microbiological investigation of male partners in infertile couple can be useful to detect the male urogenital tract infection, especially asymptomatic infections.

The isolation of microorganisms from seminal fluid especially of infertile men had been widely reported^{6,7}. It is always recommended that microbiological study of semen can be performed in asymptomatic infertile men with leukocytospermia. Aerobic and anaerobic culture of semen can detect a wide range of urogenital pathogens⁸. The most widely studied genital microorganism in relation to male infertility is *Escherichia coli*, which is also the principal microorganism that causes prostatitis and epididymitis. Infections in the reproductive tract of infertile men have been acknowledged for decades. Until recently, the condition of leukocytospermia was used as an indicator of genital tract infection⁹. However, a relatively large number of men who attend fertility clinics exhibit leukocytospermia without symptoms of genital infections, indicating that there is not a necessary relationship between infections in the genital tract and the amount of leukocytes or antisperm antibodies in semen¹⁰. This study was suggested and designated to Investigate the relationship between bacteriospermia and leukocytospermia in infertile male with

Asthenospermia and Studying some of the virulence factors and antimicrobial susceptibility patterns of the isolated bacteria.

Material and Methods

Patients: Asthenospermic seminal fluid specimens were collected from (100) infertile males. The asthenospermic specimens were divided into two group according to the presence of leukocytes in their specimens (leukocytospermia):

First group: this group included 70 asthenospermic specimens with leukocytospermia ($>1 \times 10^6$ pus cell/ml of seminal fluid).

Second group: this group included 30 asthenospermic specimens without leukocytospermia ($<1 \times 10^6$ pus cell/ml of seminal fluid).

Infertile male age rang from (25-44) years with mean age of (32.11) years. Abstinence time range from (72-120 hrs.). The specimens of patients who treated with antibiotic were excluded.

Methods: Seminal fluid specimens were collected from infertile patients by masturbation, under aseptically conditions. They were also asked to pass urine first and then wash and rinse hands and penis before the specimens were collected¹¹. The specimens were collected into clean wide-mouthed 15ml sterile plastic vials and incubated at 37°C for 30 minutes for liquefaction and then seminal fluid analysis (SFA) was done to diagnose asthenospermia and leukocytospermia. Swabs were inserted into the specimens and then directly inoculated on blood agar, chocolate agar and MacConkey agar. All plates were incubated aerobically at 37°C for 24-48 hrs.

Seminal fluid analysis (SFA): In this experiment SFA method was used to investigate leukocytospermia and asthenospermia . According to World Health Organization criteria asthenospermia defined as less than 50% of spermatozoa with forward progression or less than 25% of spermatozoa with rapid progression within 60 min after semen collection. Leukocytospermia was defined as more than 1×10^6 pus cell/ml of seminal fluid¹¹.

According to the diagnostic procedures recommended by Collee and his colleagues (1996)¹²; MacFaddin (2000)¹³ and Forbes and his colleagues (2007)¹⁴, the isolation and identification of G+ve and G-ve bacteria associated with bacteriospermia in asthenospermic patients were done.

Virulence factors tests: Blood agar medium was streaked with a pure culture of bacterial isolate to be tested and incubated at 37°C for 24-48 hrs. The appearance of a clear zone surrounding the colony is an indicator of β - hemolysin while the greenish zone is an indicator of α - hemolysin¹⁴. Haemagglutination test (HA) was performed to show the ability of bacterial isolates to produce colonization factors antigen (CFA). Lipase test was carried out in egg-yolk agar medium to determine the ability of microorganisms to produce lipase enzyme. After inoculation of the medium agar,

plates were incubated for overnight at 37°C. The appearance of opaque pearly layer around the colonies indicated for a positive result¹². Antimicrobial susceptibility test was performed according to CLSI (2010)¹⁵.

Statistical analysis: The χ^2 (Chi-square) test was used for statistical analysis. $P < 0.01$ was considered to be statistically significant.

Results and Discussion

Asthenospermia and leukocytospermia: One hundred asthenospermic seminal fluid specimens were diagnosed using seminal fluid analysis (SFA). Motile spermatozoa in all specimens were ranged 10-40% with mean motile spermatozoa (25%) and this result revealed asthenospermia according to world health organization criteria. Asthenospermic seminal fluid specimens were divided into two groups according to leukocytospermia, 70 specimens, first group, who had leukocytospermia and 30 specimens, second group, who had no leukocytospermia. White blood cells (WBCs) in seminal fluid specimens were counted and the results showed that, all specimens of first group had more than 1×10^6 pus cell/ml of seminal fluid revealed to leukocytospermia which indicates an infection¹¹, while all specimens of second group had no leukocytospermia as shown in table.1.

Bacterial isolates from asthenospermic specimens: The results of this experiment showed that 61(87.1%) specimens of first group revealed positive bacterial culture as shown in table.1 whereas 9(12.9%) specimens of first group showed no bacterial growth even after 48 hours, which may be due to the presence of another type of causative agents that might need special technique for their detection such as viruses, *Chlamydia* or *Mycoplasma*. These results were corresponding to those results being reported by Shefi and Turek¹⁶. However the results were higher than those reported by Jiao and his colleagues¹⁷, who found that (5-15%) of samples, gave positive culture. All specimens of second group gave negative bacterial culture. The results in table.1 were statistically analyzed by using χ^2 test showed that there was a strong relationship between the bacteriospermia and asthenospermia ($P < 0.01$). This result agreed with that result being reported by Golshani and his colleagues¹⁸ who declared that semen specimens of infertile men, especially those contain high number of *E. coli* and *Enterococci* isolates, had high rate of non-motile and morphologically abnormal sperms. Philip and Folstad¹⁹ confirmed that there was a significant positive effect of antibiotic treatment for the following sperm parameters: sperm volume, sperm concentration, sperm motility, and sperm morphology. Antibiotic treatment also significantly reduced the number of leukocytes in ejaculates of male infertility patients. Thus, in general, males treated with antibiotics were relieved from leukocytospermia and produced ejaculates of high quality. Also there was a strong relationship between bacteriospermia and leukocytospermia ($P < 0.01$).

Table-1
Illustration of asthenospermia, leukocytospermia and bacteriospermia

Cases		Specimens	
		First group n(%) n=70	Second group n(%) n=30
Asthenospermia		70(100%)	30(100%)
Leukocytospermia	Positive	70(100%)	0.0
	Negative	0.0	30(100%)
Bacteriospermia	Positive	61(87.1%)	0.0
	Negative	9(12.9%)	30(100%)

Table-2
Distribution of bacterial isolates from patients with asthenospermia according to the isolates

Bacterial species		Single isolates n	Mixed isolates n	Total isolates n (%)	Total n (%)
CoNS	<i>S. saprophyticus</i>	14	*4	25 (35.7)	44(62.9)
	<i>S. epidermidis</i>	7	0		
<i>S. aureus</i>		14	5	19(27.2)	26(37.1)
<i>Escherichia coli</i>		9	**3	12(17.1)	
<i>Enterobacter aerogenes</i>		6	2	8(11.4)	
<i>Acinetobacter</i> spp.		4	0	4(5.7)	
<i>Moraxella</i> spp.		2	0	2(2.9)	
Total		56	14	70 (100)	100%

*Four isolates of *S. saprophyticus* were mixed with Four isolated of *S. aureus*. **Three isolates of *E. coli* were mixed with one isolate of *S. aureus* and two isolates of *E. aerogenes*

A total of (70) bacterial isolates were obtained from the (61) seminal fluid specimens in which gram positive bacteria constituted 44(62.9%) of the total isolates and were considered as the largest etiological agent of bacteriospermia compared with gram negative bacteria which constituted 26(37.1%) as indicated in table-2 and this might be due to the fact that grams positive bacteria are commensals of mucosal surfaces of urogenital tract and these results were similar to those results being reported by Chimura and Saito²⁰ who found that G+ve bacterial strains constituted (78.4%), while G-ve bacterial strains constituted (21.6%).

Pathogenicity of bacteria in asthenospermic patients: The present study showed that asthenospermia were caused by 70 bacterial isolates Table-2. Coagulase negative staphylococci (CoNS) represented by *S. epidermidis* and *S. saprophyticus* which constituted 25(35.7%), *S. aureus* constituted 19(27.2%) were predominant in causative microorganism of bacteriospermia followed by *E. coli* 12(17.1%). However, each of the following bacteria *E. aerogenes*, *Acinetobacter* spp. and *Moraxella* spp. constituted 8(11.4); 4(5.7) and 2(2.9) respectively.

CoNS organisms were the most common bacterial group isolated from seminal fluid infections (35.7%); CoNS infections in the present study were less than those reported by other researchers²¹ who found that these infections constituted (50-89%), but they were more than those reported by Virecoulon F.

et al²², who reported that seminal fluid infections caused by CoNS were constituted (15.7%).

The high percentage of CoNS infections may be due to that they are common contaminant of skin and urethral meatus, and also their ability to resist antibiotics commonly used in medical therapy. These commensals bacteria may have a role as opportunistic pathogens in the presence of weakened local tissue defense when immunosuppressive agents were used, and the antibiotics had been associated with emergence of opportunistic infection by microorganisms not previously regarded as pathogenic bacteria²³.

S. aureus was the second in occurrence in seminal fluid specimens, which constituted 19(27.2%). This was in line with reports from other studies^{24,25}. *S. aureus* had detrimental effect of spermatozoa resulted from damage of sperm membrane lipids²⁶. The pathogenesis of *S. aureus* was attributed to the combined effects of extracellular factors and toxins, together with invasive properties such as adherence, biofilm formation, and resistance to phagocytosis²⁷. *S. aureus* may inherent nature of developing resistant strains for antibiotics. *S. aureus* also contains teichoic acid and lipoteichoic acid, capsular material which facilitated the adherence of these bacteria to epithelium of urogenital tract²⁸. The detection of staphylococci from seminal fluid specimens was documented. It was found that staphylococci involved in the pathogenesis of chronic pelvic pain syndrome (CPPS)²⁹. They were identified in focal colonies adherent to the prostatic duct walls³⁰.

Table-3
Virulence factor of bacterial isolate

Bacteria	Virulence factor			
	Hemolysin production	Lipase production	*CFA I	**CFA III
<i>S. aureus</i>	19 (100%)	15 (78.9%)	10 (52.6%)	19 (100%)
CoNS	18 (72%)	7 (28%)	8 (32%)	25 (100%)
<i>E. coli</i>	5 (41.7)	9 (75)	8 (66.7)	12 (100)
<i>Enterobacter aerogenes</i>	4 (50)	7 (87.5)	6 (75)	8 (100)
<i>Acinetobacter spp.</i>	0 (0.0)	0 (0.0)	2 (50)	4 (100)
<i>Moraxella spp.</i>	0 (0.0)	1 (50)	1 (50)	2 (100)

Results of this study also found that (37.1%) of bacteriospermia were caused by gram negative bacteria. *E. coli* represented the common gram negative bacteria isolated from seminal fluid specimens. They accounted for (17.1%) of total bacterial isolates of asthenospermic patients. This result was close to the finding by other researchers^{24,31}. In other studies *E. coli* isolates were found to be less than 10%^{21,25}. Immobilizing effect of certain bacteria, particularly *E. coli* on spermatozoa had been demonstrated, and this was the mechanism responsible for the asthenospermia resulted from bacteriospermia. Also, *E. coli* has the ability to cause sperm membrane lipid damage²⁶.

The other group of gram negative bacteria isolated from seminal fluid specimens were *E. aerogenes* (11.4%), *Acinetobacter spp.* (5.7) and *Moraxella spp.* (2.9%). This result was the highest of those reported by other studies as in Alwash (2006)³². *E. aerogenes* possess many factor that facilitate their pathogenicity as endotoxin, which have deleterious effect on seminal fluid; capsules and adhesion proteins that support their attachment to mucosal surfaces of urogenital and also have the ability of resistance to multiple antimicrobial agents¹⁴.

Virulence factors of the bacterial isolates: The factors that determine the initiation, development, and outcome of an infection involve a series of complex and shifting interaction between the host and the parasite, which can vary with different infecting microorganisms. Virulence factors of the bacterial isolates demonstrated in this work included coagulase, hemolysin, capsule, siderophore, bacteriocin, lipase and extracellular protease production as well as colonization factor antigens (CFA/I, and CFA/III).

Microorganisms evolve a number of mechanisms for the acquisition of iron from their environments. One of them is the production of hemolysins, which acts to release iron complexed to intracellular heme and hemoglobin. Another mechanism for iron acquisition is to produce siderophores which chelate iron with a very high affinity and which compete effectively with transferrin and lactoferrin to mobilize iron for microbial use³³. The results of this study revealed that all isolates of *S. aureus* were able to expressed β -hemolytic mode on blood agar. Among CoNS isolates only 18(72%) exhibited α -hemolytic pattern, while the rest CoNS isolates were γ -hemolytic (non hemolytic) pattern, which no color change around the bacterial

colonies Table-3. This agreed with the result mentioned by Dinges and his colleagues (2000)³⁴. The production of hemolysin by *S. aureus* is well known and considered as a main virulence factor for these bacteria and it associated with increased severity of infections³⁵. In G-ve, bacterial isolates five isolates of *E. coli* and four isolates of *E. aerogenes* displayed β -hemolytic pattern. The other G-ve isolates demonstrated γ -hemolytic pattern (table 3-6). Iron can increase disease risk by functioning as a readily available essential nutrient for invading microbial and neoplastic cell. To survive and replicate in hosts, microbial pathogens must acquire host iron. Highly virulent strains possess exceptionally powerful mechanisms for obtaining host iron from health hosts³⁵.

Production of lipase were detected among bacterial isolates and the results showed that 15(78.9%) of *S. aureus* and 7(28%) of CoNS isolates were capable of lipase production (table 3-5). Results of lipase production test in G-ve bacterial isolates revealed that 9(75%) of *E. coli*, 7(87.5%) of *E. aerogenes* and 1(50%) isolate of *Moraxella spp.* were lipase producer (table 3-6). Host cell membranes contain lipids in their components; lipase enzyme will destroy these elements and aids the pathogen to penetrate the host tissue to develop the infections³⁶.

All isolates were tested for their ability to produce colonization factor antigens type (CFA/I) and (CFA/III). The results revealed that all G+ve isolates were able to produce (CFA/III) and 10(52.6%) of *S. aureus*, 8(32%) of CoNS isolates were capable to produce (CFA/I) as shown in table (3-5). These factors are considered primary factors, which cause adhesion of bacteria to the target host cell, and their presence indicates that the bacteria contain cell surface fimbrial antigens. Detection of CFA in G-ve bacterial isolates were done and the results indicated presence of (CFA/III) in all G-ve isolates, while (CFA/I) were found in 8(66.7%) of *E. coli*, 6(75%) of *E. aerogenes*, 2(50%) of *Acinetobacter spp.* and 1(50%) of *Moraxella spp.* isolates (table 3-6). The (CFA/I) contributed and aided the bacteria to adhere and multiply within eukaryotic cells. Bacterial adherence to host tissues is a complex process that, in many cases, involves the participation of several distinct adhesions, all of which may act at the same time or at different stages during infection. Many pathogenic bacteria displayed polymeric adhesive fibers termed "pili" or "fimbriae" that facilitated the initial attachment to epithelial cells and subsequent successful colonization of the

host³⁷. Pili are virulence factors that mediate interbacterial aggregation and biofilm formation, or mediate specific recognition of host-cell receptors (Jonson *et al.*, 2005). It is clear that pili play similar biological roles for commensals bacteria because they also have to colonize specific niches and overcome the host's natural clearing mechanisms. It is thought that commensal and some pathogenic *Escherichia coli* strains use type I pili or curli to colonize human and animal tissues³⁸.

Effect of some antibiotics on bacterial isolates: figure (3-1) displays the resistance of all G+ve and G-ve bacterial isolates to amoxicillin and amoxicillin-clavulanic acid. The results revealed that all bacterial isolates showed high resistance (75% - 100%) to amoxicillin, but less resistance to amoxicillin-clavulanic acid (47.4% - 75%). Among G+ve bacterial isolates the resistance of *S. aureus* and CoNS isolates to amoxicillin were (100%) for both. These results are agreeable with results obtained by Dan³⁹ who confirmed that the resistance of CoNS isolates to β -lactams was mediated by β -lactamase enzymes production under chromosomal control. Both *S. aureus* and CoNS isolates exhibited low level of resistance toward amoxicillin-clavulanic acid 9(47.4%), 13(52%) respectively. Addition of clavulanic acid can inhibit the action of β -lactamases enzyme⁴⁰. These results matched those obtained by Romolo and his colleagues⁴¹ who pointed out that the uropathogens resistance to amoxicillin was as high as to amoxicillin-clavulanic acid. The use of clavulanic acid decreased the resistance of bacteria to β -lactame antibiotics. The mechanism of this resistance is mostly due to either production of β -lactamases that hydrolyze β -lactame ring which was controlled by plasmid or chromosomal regulation, or lack of penicillin receptors on cell wall and/or alteration in their permeability to β -lactam antibiotics and preventing the uptaking

of antibiotics⁴². Among G-ve bacterial isolates the resistance of *E. coli* to amoxicillin was 12(100%) which was higher than to amoxicillin-clavulanic acid 8(66.7%). This result was in line with other results reported by Dulawa and his group⁴³ who observed an upward trend in the resistance of *E. coli* to amoxicillin/ampicillin and this resistance is predominantly caused by plasmid-encoded β -lactamase TEM-1; these enzymes preferentially hydrolyze penicillin, which was sensitive to β -lactamase inhibitors such as clavulanic acid. So, addition of clavulanic acid can inhibit the action of these enzymes and only 70% were resistant to amoxicillin-clavulanic acid⁴⁰. Generally, resistance to beta-lactam antibiotics in G-ve bacteria can be due to four mechanisms: Decreased permeability of the drug into the cell, hydrolysis of the drug by β -lactamase, decreased affinity of the target penicillin-binding proteins (PBPs), or by pump-mediated resistance¹⁴.

The resistance of *Acinetobacter* to amoxicillin was (100%) and this result was higher than those reported by Alwash B.H.³² and Al-Shukri M.S.⁴⁴ who clarified that the resistance rate of uropathogenic *Acinetobacter* to amoxicillin was (63.6%) and (80%) respectively. Enzyme resistance was resulted from the ability of *Acinetobacter* to produce β -lactamase^{14, 44}. Only three isolates of *E. aerogenes* were resisted to amoxicillin and this results in agreement with those results being reported by other researcher⁴⁵. Also Dumarche and his colleagues⁴⁶ reported that all *E. aerogenes* isolates which produce (ESBL) had one or more of plasmids which carry multiresistance genes. Two isolates of *Moraxella* spp. were resistant to amoxicillin and amoxicillin-clavulanic acid. Mechanism of resistance exhibited by *Moraxella* was similar to those of *Acinetobacter*. Varon and his researchers (2000)⁴⁷ found that *M. catarrhalis* were fully sensitive to amoxicillin.

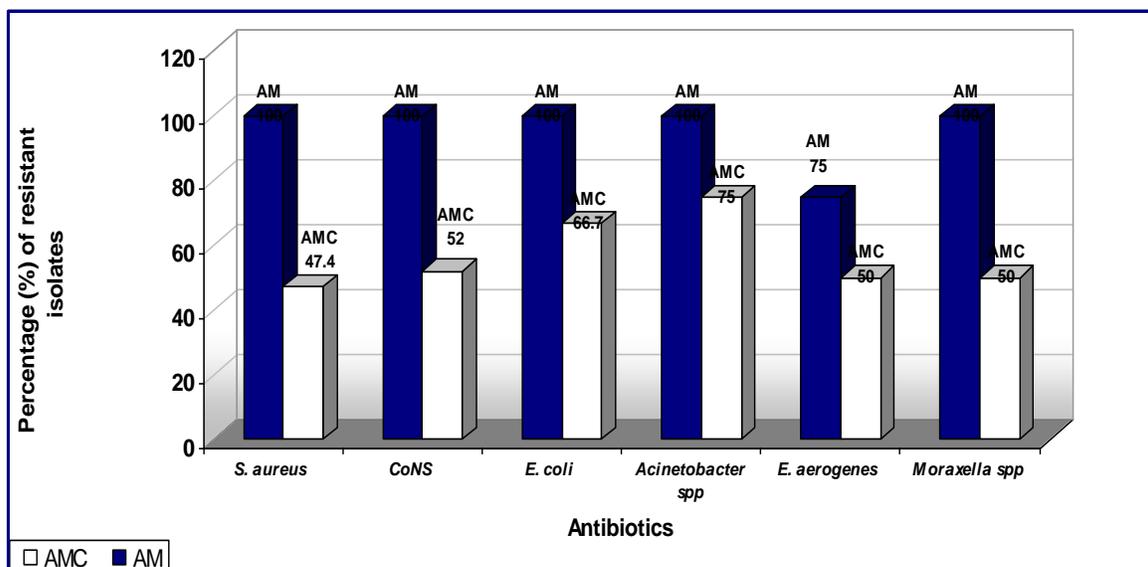


Figure-1
 Resistance of bacterial isolates to amoxicillin and amoxicillin clavulanic acid.
 AM: amoxicillin, AMC: amoxicillin-clavulanic acid

Resistance of bacterial isolates to the cephalosporins was studied. Figure (3-2) reveals variable levels of resistance to different generations of cephalosporins. *S. aureus* resistance to cefamandole (2nd generation), ceftizoxime, ceftazidime (3rd generation) and cefepime (4th generation) were 73.7% , 84.2% , 100% and 68.4% respectively .This result revealed that *S. aureus* exhibited low level of resistance to 4th generation cephalosporin than other cephalosporins. This result agreed with Brooks and his colleagues⁴⁸. CoNS isolates displayed low level of resistance to cephalosporins (56%-80%) than those exhibited by *S. aureus*. Resistance to cephalosporins mediated by cephalosporinase production¹⁴. All G-ve bacterial isolates were fully (100%) resistance to cefamandole (second-generation cephalosporin) except *E. coli* and *E. aerogenes* (91.7%, 75%) respectively. *S. aureus* and CoNS isolates exhibited less level of resistance to cefamandole than G-ve isolates. All isolates of G-ve bacteria exhibited nearly similar levels of resistance to cephalosporins. *Acinetobacter* spp. isolates were fully resistance to cephalosporins, also six isolates of *E. aerogenes* were resistant to all cephalosporins. This resistance may be resulted from combination of unusually restricted outer membrane permeability and chromosomally encoded β -lactamase. This agreed with results mentioned by Bisiklis and his workers⁴⁹.

in *Moraxella* spp. which displayed resistance to both of these antibiotics which might be due to the low number of *Moraxella* isolates in the present study. However, the result was in accordance with those reported by Watanabe and his colleagues (2000)⁵⁰ and Nomura and Nagayama⁵¹. Imipenem and meropenem are broad-spectrum carbapenems antibiotics. Beta-lactam rings of these antibiotics are resistant to hydrolysis by most beta-lactamases and the activity of meropenem against most clinical isolates was comparable with imipenem. These antibiotics pass through the outer membrane of G-ve bacteria via the water filled porin channels to reach their targets, penicillin binding proteins¹⁴. Deletion or diminished production of these outer membrane proteins (porins) decreases outer membrane permeability of some G-ve bacteria for diffusion of these antibiotics and decreases susceptibility to imipenem and meropenem⁴⁸. Generally a distinct difference was present between β -lactamase production by G+ve and G-ve bacterial isolates, for example β -lactamase produced by staphylococci were excreted into the surrounding environment where the hydrolysis of β -lactams takes place before the drug can bind to PBPs in the cell membrane. In contrast, β -lactamase produced by G-ve bacteria remained intracellular in the periplasmic space where they were strategically positioned to hydrolyze β -lactams as they transverse the outer membrane through water filled, protein lined porin channels¹⁴.

Figure (3-3) showed that all bacterial isolates exhibited high sensitivity to imipenem and meropenem (carbapenems) except

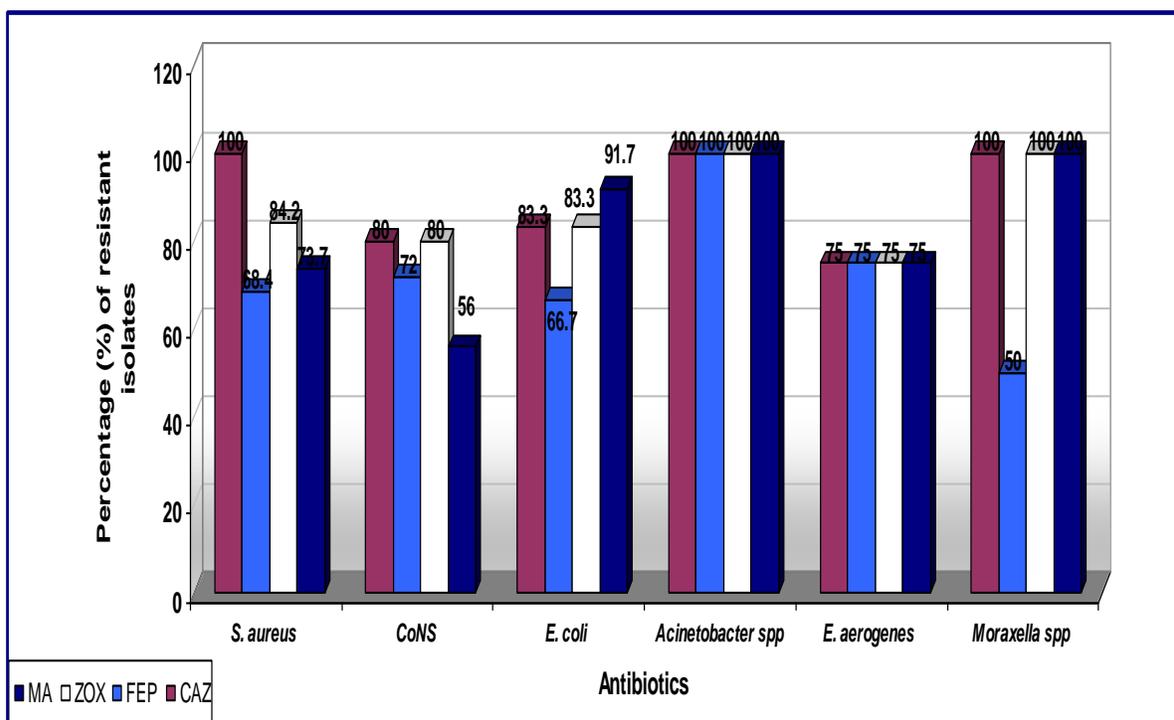


Figure-2
 Resistance of bacterial isolates (gram positive and gram negative) to cephalosporins.
 MA: cefamandole, ZOX: Ceftizoxime, FEP: Cefepime, CAZ: ceftazidime

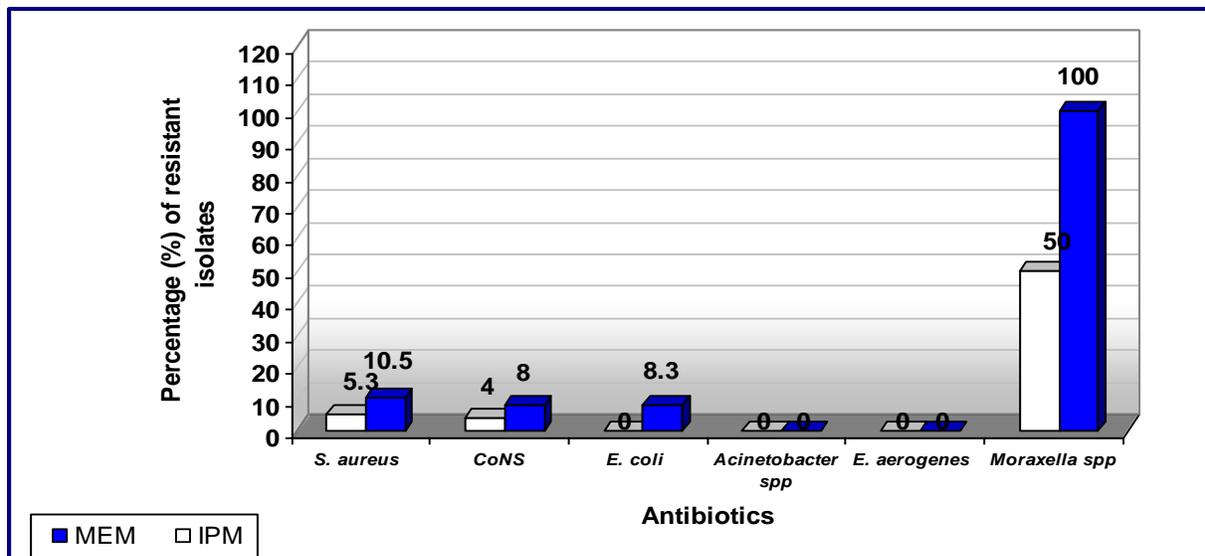


Figure-3
 Resistance of bacterial isolates to carbapenems.
 MEM: meropenem, IPM: imipenem

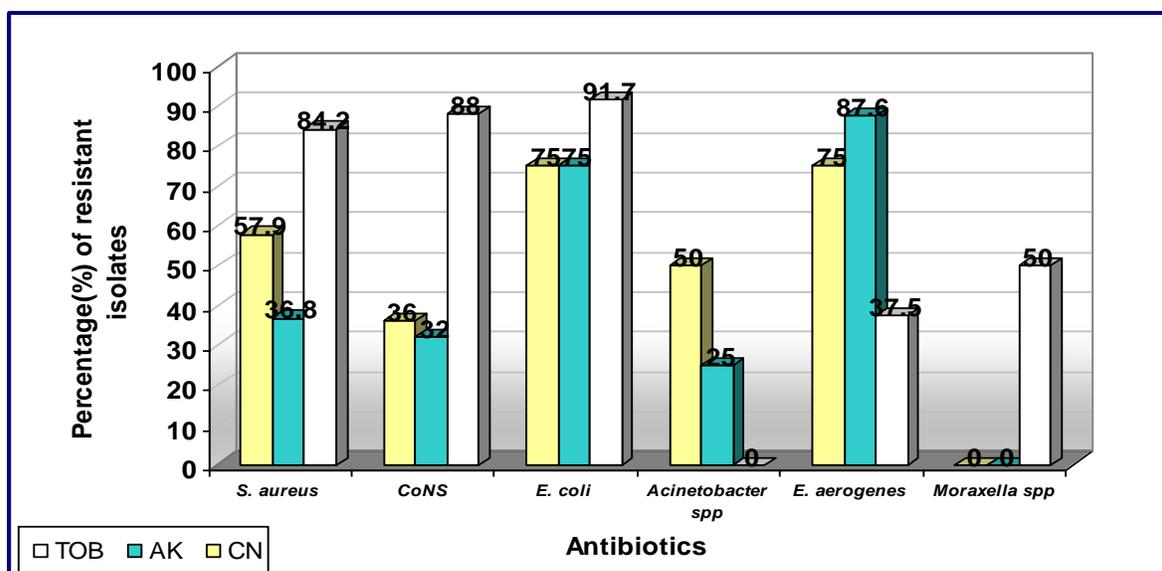


Figure-4
 Resistance of bacterial isolates to aminoglycosides.
 TOB: tobramycin, AK: amikacin, CN: gentamycin.

Resistance of the bacterial isolates to aminoglycosides were established in figure -4. The results revealed that *S. aureus* and CoNS isolates showed similar status of resistance to gentamycin (84.2%, 88%) respectively. The mechanism of aminoglycosides resistance by staphylococcal isolates is enzymatic modification, in which modifying enzymes alter various sites on the aminoglycosides molecule so that the ability of drug to bind the ribosome and halt protein synthesis was greatly diminished or lost. This result was agreed with Alwash B.H.³², who found that (80%) of *Staphylococcus spp.* isolates were exhibited resistance

to gentamycin. However, Khorshed (2005)⁵² reported that *Staphylococcus spp.* isolated from UTI were very sensitive to gentamycin (low level of resistance 15%). *S. aureus* and CoNS gave low level of resistance to amikacin, (36.3%, and 32% respectively) and also to tobramycin (57.9%, 36% respectively) when compared with their resistance to gentamycin.

Resistance to gentamycin had been identified in CoNS isolates. Moreover, CoNS may function as a reservoir for antibiotic resistant genes to *S. aureus*. Among G-ve bacterial isolates,

91.7% of *E. coli* isolates were resistant to tobramycin. Only (75%) of *E. coli* isolates were resistant to amikacin and gentamycin. These results agreed with those reported with Al-Muhanna⁵³ and Al-Nuaimi⁵⁴, who found that *E. coli* was fully resistant to amikacin. However, this result disagreed with other local studies as given by Alwash B.H.³² who found that *E. coli* isolated from patients with urinary tract infections (UTI) and from those with prostatitis exhibited low level of resistance to amikacin (7.7%-25%). This resistance could be interpreted depending on the fact that many strains of *E. coli* have acquired plasmids conferring resistance to one or more than one type of antibiotics, therefore antimicrobial therapy should be guided by laboratory result test of sensitivity⁵⁵. *Acinetobacter* spp. isolates were fully sensitive to tobramycin, but they showed low resistance to amikacin (1/4) and (2/2) of them were resist gentamycin. This result was in line with those documented by Al-Shukri M.S.⁴⁴ and Al-Hamawandi J.A.⁵⁵ who observed that *Acinetobacter* was resistant to gentamycin and this resistance was produced through alteration of the ribosomal target site, and production of aminoglyside-modifying enzyme. Moreover, Hpa established that resistance of uropathogenic *Acinetobacter* to gentamycin and amikacin were 43% and 5% respectively. Concerning *E. aerogenes* resistance of aminoglycosides, the results revealed that (6/8) of *E. aerogenes* isolates were resistant to gentamycin (7/8) were resistant to amikacin and (3/8) of them were resisted tobramycin.

Enterobacter spp. resistance to gentamycin was (75%). Park and his colleagues had stated that the resistance rate of *Enterobacter* spp. to gentamycin was (33.3%) while it was (54%) for amikacin and that differ from the results in the present study. The mechanism of *E. aerogenes* resistance to aminoglycosides was mediated by the production of more than one type of aminoglycosidases located on the R plasmid. Other mechanism was post transcriptional modification of 16S rRNA which can

confer high level resistance to all aminoglycosides except streptomycin in G-ve human pathogens including *E. aerogenes*⁵⁶. *Moraxella* spp. isolates were fully sensitive to gentamycin and amikacin. Only (1/2) of *Moraxella* spp. isolates were resist to tobramycin.

In the present study the results of fluoroquinolones (ciprofloxacin and norfloxacin) resistance are displayed in figure (3-5). G+ve isolates exhibited low resistance to both ciprofloxacin and norfloxacin, (42.1%) of *S. aureus* and (12%) of CoNS isolates were resist to ciprofloxacin, while resistance to norfloxacin was (36.8%, 32%) respectively.

This result agreed with other local studies as given by Khorshed P.A.⁵² who found that only (20%) of *staphylococcus* spp. isolated from patients with UTI were resistant to ciprofloxacin. Also, Alwash³² found that (33.3%) of *S. aureus* and (11.1%) of CoNS isolates were resisted ciprofloxacin. Similarly, Rachid and his group (2000)⁵⁷ observed that there were an increased number of strains resistant to ofloxacin and ciprofloxacin. Kurt and Naber (2001)⁵⁸ document that the ciprofloxacin was the first choice for seminal fluid tract infection. Moreover, Donnell and Gelone, (2000)⁵⁹ reported that the resistance to flouroquinolones was through chromosomal mutations or alterations affecting the ability of fluoroquinolones to permeate the bacterial cell wall. Fortunately, separate isomerases were required to produce this form of resistance⁴¹. Forbes and his colleagues¹⁴ stated that staphylococci had two mechanisms to resist flouroquinolones; the first one was efflux mechanism in which an activation of efflux pump that removes flouroquinolones before intracellular concentration sufficient for inhibiting DNA metabolism can be achieved. The other mechanism (target alteration) included changes in DNA gyrase subunits decrease ability of flouroquinolones to bind this enzyme and interfere with DNA processes.

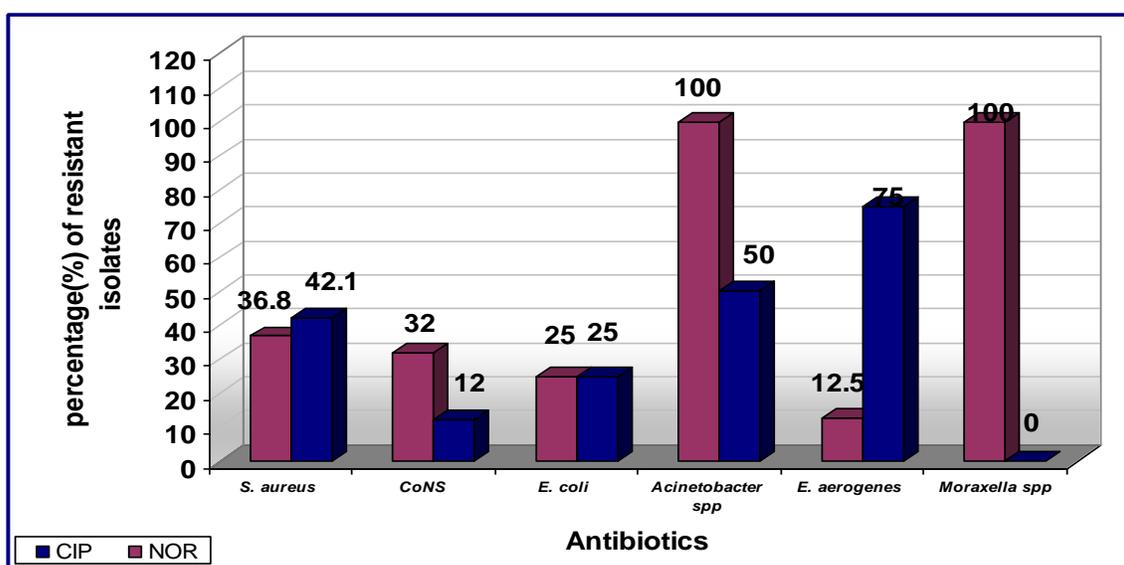


Figure-5
 Resistance of bacterial isolates to Fluoroquinolones. CIP: Ciprofloxacin, NOR: norfloxacin

Flouroquinolones resistance among G-ve bacterial isolates were also studied. (25%) of *E. coli* isolates were resistant to both ciprofloxacin and norfloxacin. This result was in line with results obtained by (32,52) who found that, the resistance rate of *E. coli* to ciprofloxacin was (36.4%), and differed from Klligore and his colleagues⁶⁰ who demonstrated that the resistance rate of uropathogenic *E. coli* to ciprofloxacin was (0.4%, 13%) respectively. (4/4) and (2/4) of *Acinetobacter* spp. isolates were resistant to norfloxacin respectively. Resistance of G-ve isolates to flouroquinolones occurred by one of the two strategies, either by alteration in the outer membrane led to diminishes uptake of drug, or by changes in DNA gyrase subunits which decreases ability of flouroquinolones to bind this enzyme and interfere with DNA processes¹⁴. In addition to that, Jacoby and his collagueus (2006)⁶¹ stated that *Enterobacter* had plasmid-mediated quinolones resistance gene which confer their resistance to the flouroquinolones.

From the data gathered above we can conclude that, There is a significant relationship between asthenospermia and bacteriospermia. *Staphylococcus aureus* (CoNS) represented by *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, *Escherichia coli*, *Enterobacter aerogenes*, *Acinetobacter* spp. and *Moraxella* spp. seem to be the most common bacteria associated with bacteriospermia. There is a significant relationship between leukocytospermia and bacteriospermia and leukocytospermia can be used as predictor of bacteriospermia. The bacterial isolates associated with bacteriospermia showed resistance to many antibiotics but they were highly susceptible to imipenem, meropenem and ciprofloxacin. All bacterial isolates in this study have the ability to possess more than one virulence factors such as coagulase, capsule, siderophore, hemolysin, extracellular protease, lipase and adherence factors to produce asthenospermia.

Conclusion

In conclusion, there is a significant relationship between asthenospermia and bacteriospermia. The most common bacteria closely associated with bacteriospermia are *Staphylococcus* spp., *Acinetobacter* spp., *Moraxella* spp., *E. coli*, and *Enterobacter aerogenes*. Most of these bacterial types are resistant to antibiotics but in general, they are highly susceptible to Imipenem, Meropenem, and Ciprofloxacin

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