Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 332 - 342
Received 05 March 2021; Accepted 01 April 2021.

Bacterial Profile and Resistance Patterns of Bacteriospermia among Pyospermic Patients in Hilla City, Iraq

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ABSTRACT

Background: Male genital tract infection is one of the most significant reasons for male infertility around the world. Attack of bacteria into the genital tract has been frequently revealed to be related with reduced sperm function, leading to infertility. The current study aim to investigate the bacterial and resistance profile among patient with pyosperimia. Methodology: A total of (110) seminal fluid samples from men with primary and secondary infertility disorder, who attended to the infertility clinic at Babylon Hospital for Maternity and Children and to private laboratory. were collected during the period from September 2020 to January 2021.rapidly transferred to Microbiology Lab, and standard bacterial culture method (on chromogenic agar) was performed to detect microbial agents All isolates screened by uti chromogenic agar is used as a selective medium for the isolation of urinary tract infection, also CHROM agar Orientation, offers simultaneous presumptive identification of Gram's positive and Gram's negative bacteria and yeasts on a single medium by means of distinct colony colors produced by reactions of genus- or species-specific enzymes with a suitable chromogenic substrateResults: The results of culturing showed that bacterial growth was positive only in 70/110(63.63%) samples while 40/110(36.37%) show no growth among pyospermic patients. Gram positive bacterial isolates compile 30/70(42.8%) were Enterococcus faecalis 18/70(25,71%) and Staphylococcus aureus 12/70(17.14%). Gram negative bacterial isolates compile 40/70(57.1%) were Escherichia coli 30/70(42.9%) Followed by, Enterobacter aerogenes 8/70(11.4%) and Proteus spp. 2/70(2.9%).Concern antibiotic resistance the results reveald thatE. coli show high resistance to ceftazidime and ceftriaxone (83.3%), amoxicillin-clavulanic acid (56.6%), trimethoprim and ciprofloxacin (53.3%) and ceftriaxone (50%). Resistance of Enterobacter aerogenes also show same resistance pattern of E. coli , ceftazidime and ceftriaxone (100% and 62.5% respectively), amoxicillin-clavulanic acid (62.5%), trimethoprim (50%). The 2 isolates of Proteus spp. show resistance to ceftazidime, ciprofloxacin and levofloxacin. Only 1 isolates was resist to amoxicillin-clavulanic acid , cefotaxime, ceftriaxone, amikacin and trimethoprim. The resistance of gram positive bacteria revealed that, Staphylococcus aureus show resistance: (75%) to rifampin, (66.7%) to erythromycin, (58.3%) to each of ampicillin, doxycycline, gentamycin and trimethoprim. Enterococcus faecalis show resistance to rifampin (83.3%), erythromycin (77.7%), trimethoprim (61.1%), doxycycline (61%), gentamycin (58.3%) and ciprofloxacin (55.5%). Conclusion: the current study conclude dominant of E. coli, E. arogenes, S. aureus and E. faecalis as cultarabile bacteria among bacteriospermic-pyospermic patints and seem the routine antibiotics used to treat such infections like cephalosporin, trimethprime, cirpfloxacin and rifampin and erythromycin were highly resisted by bacterial siolates.

Keywords: bacteriospermia, pyospermia, E. coli, E. aerogenes, S. aureus, E. faecalis, trimrthoprime, ciprofloxacin, rifampin

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Introduction

Bacteriospermia can be referred to presence of bacteria in seminal fluid. The infection with bacteria is one of the major and significant factors in male infertility that result in abnormal semen parameters and even lead to impairment of sperm functions and seminal tract obstruction. It has detrimental effects on sperm cell function by reducing sperm motility, viability, and abnormal morphology as well as premature acrosome reaction[1,2].The presence of bacteria in concentrations greater than 10^3bacteria/mL ejaculate is clinically regarded as a sign of an active infection and is called bacteriospermia. Bacterial infection can negatively affect different parts of the male genital tract and subsequently cause impaired spermatogenesis and male fertility[3].

However, most of the previous studies have focused on the infected organs of the male genital tract and there are no many studies that investigated the direct effect of bacteria on sperm and their mechanism of action. Interestingly, bacteria can induce different damages on sperm cells such as DNA fragmentation, cell membrane peroxidation, and acrosome impairment[4,5]. Such negative effects can be mediated by bacteria-secreted toxins and metabolites or by direct attachment of bacteria on the sperm cells and subsequent activation of signaling pathways related to oxidative stress, apoptosis, and inflammation [6-10]. These bacteria induced changes can impair semen parameters and subsequently cause infertility. Given the significant destructive effect of some bacteria on sperm function and male fertility[11,12].

Human seminal fluid hosts a microbiome including several hundreds of bacterial species per individual with various levels of abundances. Chinese study was found seminal bacterial communities were dominated by *Lactobacillus*, *Pseudomonas,* and *Prevotella*, respectively and have advert effects of sperm quality[13]. The most prevalent pathogens including *Chlamydia trachomatis, Ureaplasma urealyticum, Neisseria gonorrhoeae,* and *Mycoplasma hominis*. *Escherichia coli* are considered the most common nonsexually transmitted urogenital tract microbes [14,15]. Viridans streptococci, *S. aureus, S. saprophyticus, P. vulgaris, P. mirabilis*, *Enterobacter aerogenes*, *Acinetobacter* spp., *Moraxella* spp. and MRSA were isolated from Asymptomatic Infertile Males in Pakistan and Iraq[16-18].The accurate antibiotic treatment can relief the infection and fasten the improvement of semen quality changing from infertility to fertility. Antibiotic resistance become critically challenging for clinicians to treat patients infected with multidrug resistant (MDR), extensively resistant (XDR), or pandrug resistant (PDR) bacteria [19]. Meta-analysis study on the effects of broad-spectrum antibiotic treatment of male leukocytospermia-associated infertility showed that antibiotic treatment of leukocytospermic men, without diagnosed genital tract infections, resulted in a significant improvement of ejaculate quality, that is, an increase in ejaculate volume, sperm concentration, number of motile spermatozoa, and number of spermatozoa with normal morphology. Moreover, the amount of leukocytes in semen was also reduced [20]. The current study aims to investigate the bacterial profile and antibiotic resistance patterns among seminal fluid sample isolated from infertile patients with pyosperimia. Male urogenital tract infection (UTI) is one of the most important causes of male infertility, being associated with 8%-35% of male infertility. Pathogenic bacteria may interfere with infertility treatment involving the application of in vitro fertilization. Microorganisms might affect the spermatozoa function in different ways: (a) By direct contact on sperm cells; by the help of some organelles such as pili; causing agglutination of motile sperm, reducing ability of the acrosome reaction, and also causing alterations in cell morphology. (b) Trigger a local inflammatory reaction leading to increase in reactive oxygen species (ROS). (c) Induction of sperm autoantibodies. (d) Production of cytotoxic factors. (e) Infection treatment

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with antibiotics for long time may lead to defect in the sperm[21], and even lead to impairment of sperm functions and seminal tract obstruction [22].

Materials and Methods

Sample collection:

A total of (110) seminal fluid samples from men with primary and secondary infertility disorder, who attended to the infertility clinic at Babylon Hospital for Maternity and Children and to private laboratory. were collected during the period from September 2020 to January 2021., by masturbation, after a 3-day abstinence period. Before collecting the sample, patients must wash their hands and genital area with soap and water. Samples were collected in sterile plastic containers used for collecting of urine sample.

Exclusion Criteria

Patients should not take any antibiotic from one week before collecting a semen sample.

Culturing and Identification:

All of the samples were rapidly transferred to Microbiology Lab, and standard bacterial culture method (on chromogenic agar) was performed to detect microbial agents. All isolates screened by UTI chromogenic agar is used as a selective medium for the isolation of urinary tract infection, also CHROM agar Orientation, offers simultaneous presumptive identification of Gram's positive and Gram's negative bacteria on a single medium by means of distinct colony colors produced by reactions of genus- or species-specific enzymes with a suitable chromogenic substrate [23,24]. and confirmed by the species specific diagnostic genes of the most frequent isolates.

Antibiotic Susceptibility Test:

The Antimicrobial susceptibility testing (AST) was performed by disk diffusion method (Kirby-Bauer) according to CLSI-2019. Nineteen different antibiotics, including amoxicillin-clavulanic acid (AMC) (20/10µg), ampicillin (AM) (10µg), cefotaxime (CTX) (30µg), ceftriaxone (CRO) (30µg), ceftazidime (CAZ) (30µg), imipenem (IMP) (10µg), meropenem (MEM) (10µg), gentamicin (CN) (10µg), amikacin (AK) (30µg), erythromycin (E) (15µg), ciprofloxacin (CIP) (5µg), levofloxacin (LEV) (5µg), azithromycin (AZM) (15µg), Trimethoprim (TMP) (5µg), Doxycycline (DO) (30µg), Rifampin (RA) (5µg), vancomycin (VA) (30µg), nitrofurantion (F) (300µg). After adjustment of suspension to 0.5 McFarland, the inoculum was spreading on Muller-Hinton agar and incubated for 24 hours at 37ºC and then inhibition zone measured and compared with CLSI-2019 to interpreted as sensitive, intermediate or resistant.

DNA extraction and PCR:

The total genomic DNA were extracted using G-spinTM Genomic DNA Extraction Kit [for Bacteria] (Cat. No.: 17121) according to the manufacturer instruction(IntronBio/Korea). PCR was performed to amplify D-alanine D-alanine ligase (ddl) gene of Enterococcus faecalis and beta-glucuronidase enzyme (uidA) gene of Escherichia coli as mentioned in table (1)

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Table 1. Primer sequence and PCR conditions

F/R	Primer Sequence 5-3	Product (bp)	Annealing temp. (ºC)
ddlF	ATCAAGTACAGTTAGTCTT	941	48.7
ddlR	ACGATTCAAAGCTAACTG		
uidAF	TGGTAATTACCGACGAAAACGGC	162	60.9
uidAR	ACGCGTGGTTACAGTCTTGCG		

Ethical Approval 9
A valid consent was achieved from each patients before their inclusion in the study.

Results
The results of culturing showed that bacterial growth was positive only in 70/110(63.63%) samples while 40/110(36.37%) show no growth among pyospermic patients As shown in figure (1). Gram positive bacterial isolates compile 30/70(42.8%) were Enterococcus faecalis 18/70(25,71%) and Staphylococcus aureus 12/70(17.14%). Gram negative bacterial isolates compile 40/70(57.1%) were Escherichia coli 30/70(42.9%) Followed by, Enterobacter aerogenes 8/70(11.4%) and Proteus spp. 2/70(2.9%) as shown in table (2). The cultural characteristic on UTI chromagar was shown in figure (1). The highest prevalence bacteria , E. coli and E. faecalis identification were confirmed by species specific primer pairs (for uidA and ddl genes for E. coli and E. faecalis respectively) (figure 3 and 4). Concern antibiotic resistance the results reveald that E. coli show high resistance to ceftazidime and ceftriaxone (83.3%), amoxicillin-clavulanic acid (56.6%), trimethoprim and ciprofloxacin (53.3%) and ceftriaxone (50%) (figure 5). Resistance of Enterobacter aerogenes also show same resistance pattern of E. coli , ceftazidime and ceftriaxone (100% and 62.5% respectively), amoxicillin-clavulanic acid (62.5%), trimethoprim (50%) (figure 6). The 2 isolates of Proteus spp. show resistance to ceftazidime, ciprofloxacin and levofloxacin. Only 1 isolates was resist to amoxicillin-clavulanic acid , cefotaxime, ceftriaxone, amikacin and trimethoprim (figure 7). The resistance of gram positive bacteria revealed that, Staphylococcus aureus show resistance: (75%) to rifampin, (66.7%) to erythromycin, (58.3%) to each of ampicillin, doxycycline, gentamycin and trimethoprim (figure 8). Enterococcus faecalis show resistance to rifampin (83.3%), erythromycin (77.7%), trimethoprim (61.1%), doxycycline (61%), gentamycin (58.3%) and ciprofloxacin (55.5%) (figure 9).

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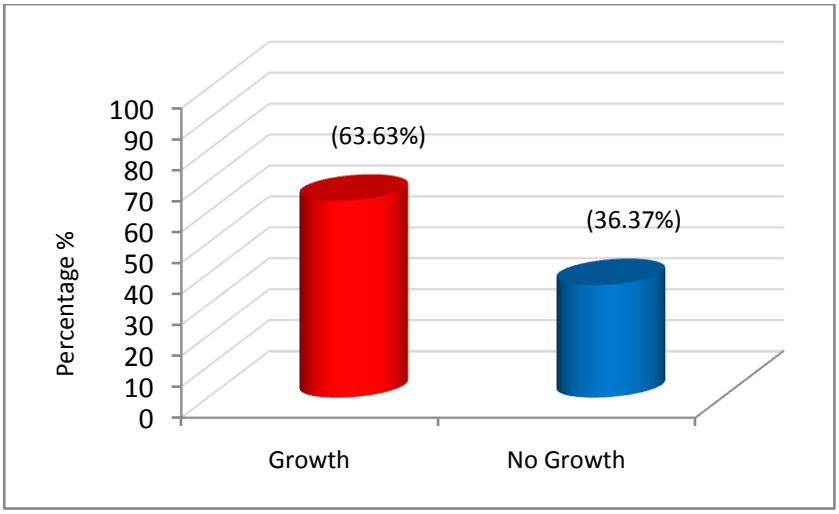

Figure 1. Bacterial growth among pyospermic patients.

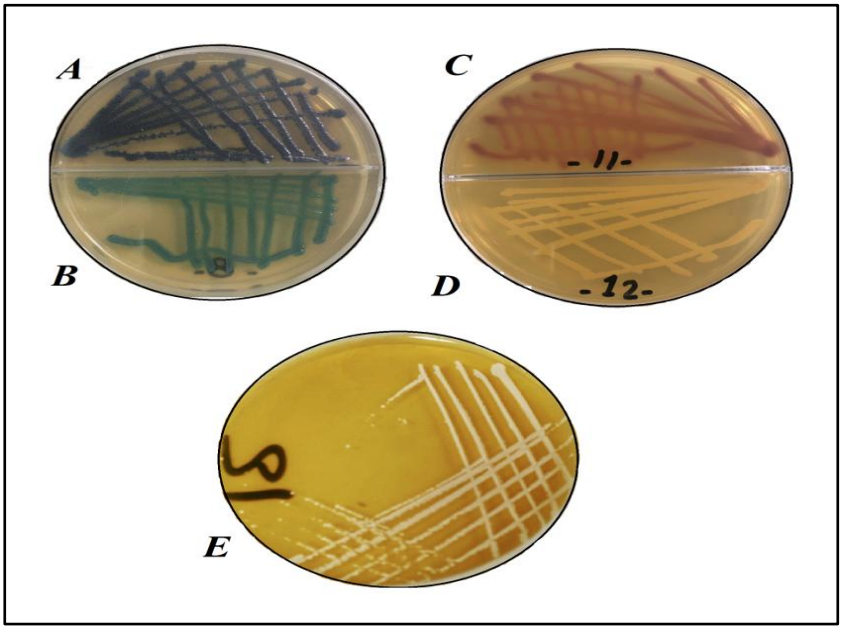

Figure 2. Bacterial isolates on UTI chromagar
A:*E. serohgenes appear dark blue on colonies UTI chromogenic agar*
B:*E. faecalis* appear trquaz on colonies UTI chromogenic agar
C: *E. coli* appear pink colonies on UTI chromogenic agar .
D:*Protus .spp light brown* on UTI chromogenic agar .
E:S.aureus appear creamy on colonies UTI chromogenic agar ..

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Table 2. Bacterial profile among pyospermic patients.

Bacterial species	**No. of isolates (%)**	
Gram-negative (40/70)	*E. coli*	30/70 (42.9 %)
	E. aerogenes	8/70 (11.4 %)
	Proteus .spp	2/70 (2.9 %)
Gram-positive (30/70)	*S. aureus*	12/70 (17.14 %)
	E. faecalis	18/70 (25,71%)

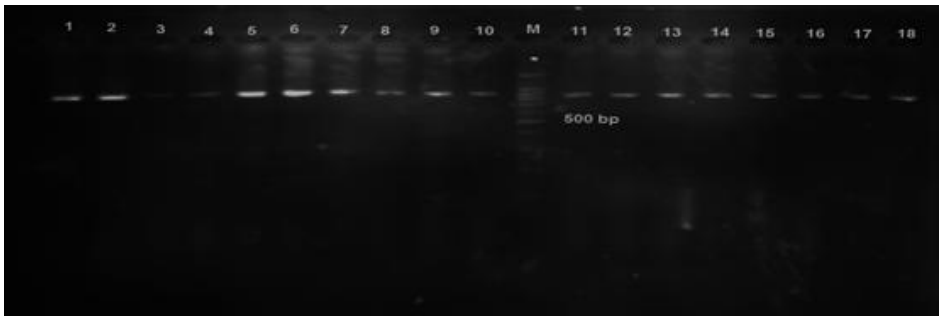

Figure 3. (1.5%) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to ddl *E. faecalis* (941bp), M represent DNA marker (100bp).

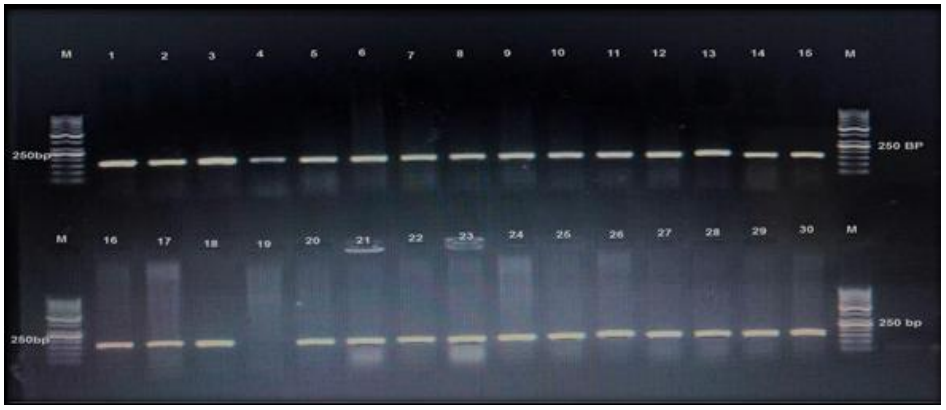

Figure 4. (1.5%) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to uidA *E. coli* amplicon (162bp), M represent DNA marker (100bp).

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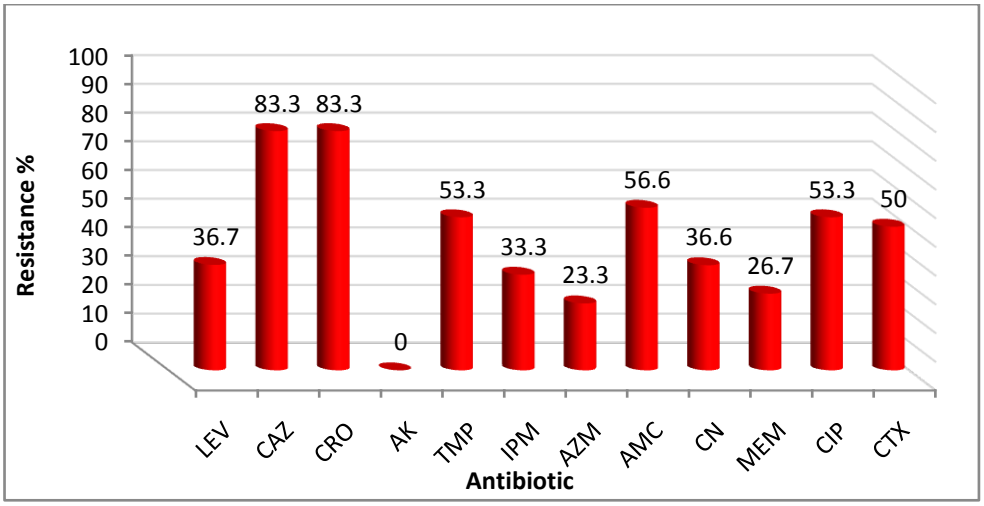

Figure 5. Antibiotic resistance among *Escherichia coli*

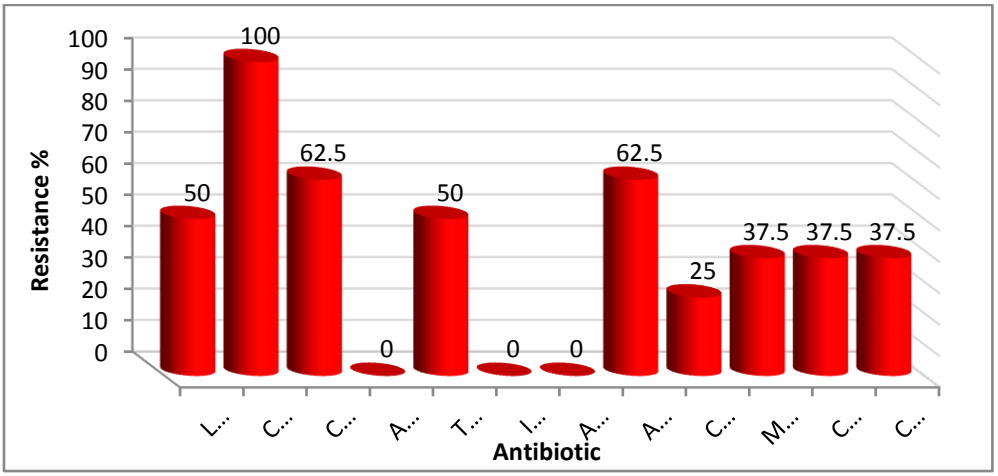

Figure 6. Antibiotic resistance among *Enterobacter aerogenes*

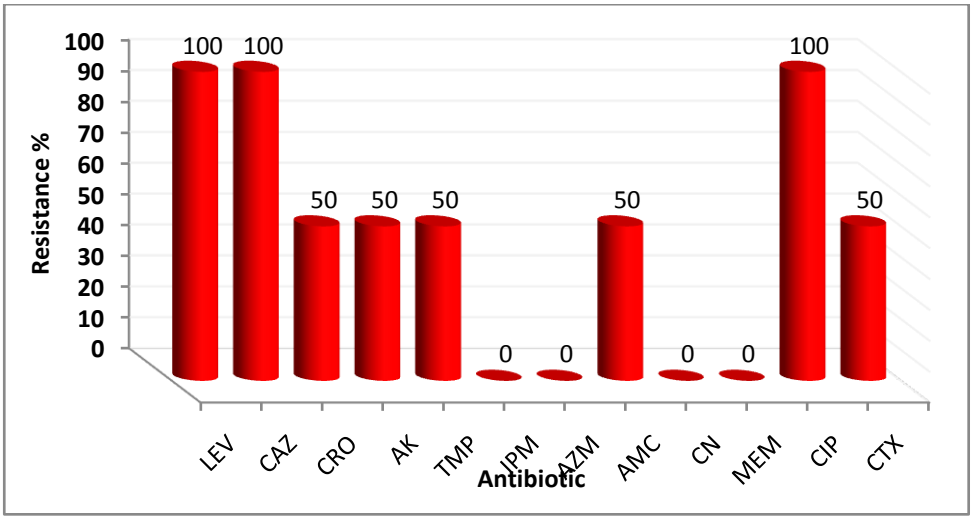

Figure 7. Antibiotic resistance among *Proteus spp.*

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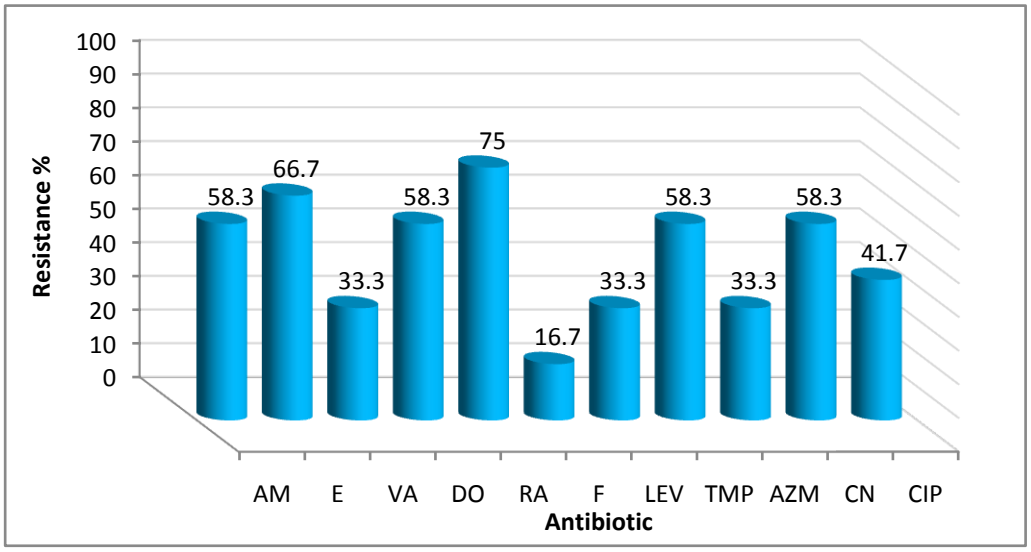

Figure 8. Antibiotic resistance among *Staphylococcus aureus*

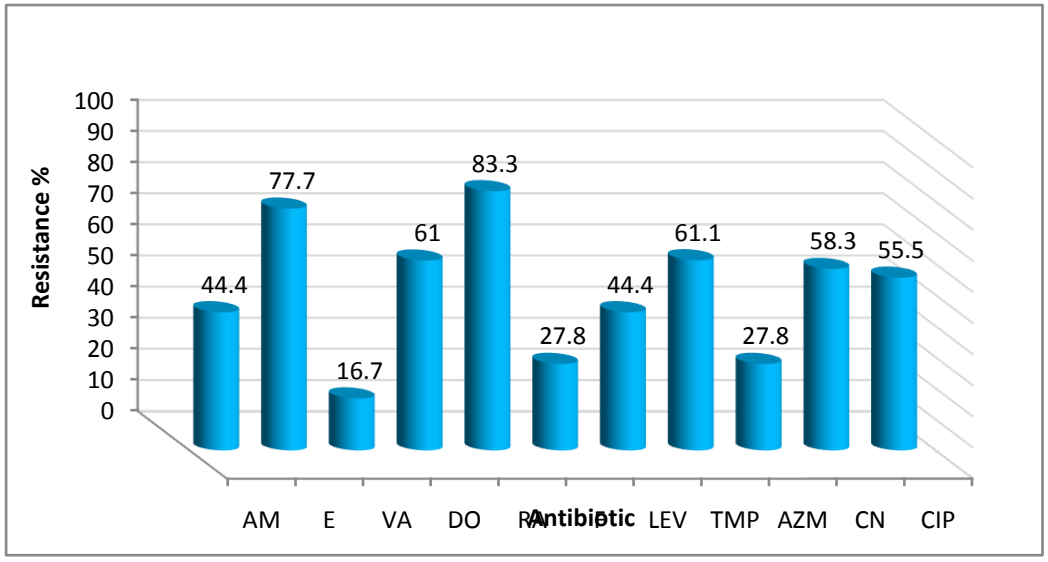

Figure 9. Antibiotic resistance among *Enterococcus faecalis*

Discussion

In our study, the significant bacterial growth was seen in 63.6% of semen specimens. Literature shows a wide variation of isolation; Enwuru, et al. (2016)[25] reported 70.4% significant bacterial growth, and similar with less prevalence rates of 42.9%, 51.7%, 52.5%, Similar prevalence rates 65.7%, 66%, and with a higher spread rate 79% were shown by Mogra et al (2009)[26]. Khadim and Al-Bermani, (2020)[27] show that Gram negative and Gram positive bacteriospermia were (64%) and (36%) respectively, which was similar to our study results. Taher et al (2019) [28] show that patients infected with Gram-negative isolates were found to be

significantly older than those with Gram-positive. Many studies sharing our results and show that E. coli, S. aureus and E. faecalis most dominant bacteria among bacteriospermic patients [29-31]. Ochsendorf [32] concluded that the urinary tract is the origin of organisms infecting the semen.

Bacteriospermia had a significant negative effect on sperm parameters; concentration, motility, progressive motility DNA fragmentation and chromatin condensation. Also, the fertilization rate decreased significantly with infected patients [29,33-35]. Among bacteriospermic patients, significant declines in sperm count, motility, and morphology have seen [32]. Ricci et al [36] found a negative influence of leukocytes on sperm function and fertilization rates as leukocytes represent the main source of reactive oxygen species in both seminal plasma and sperm suspensions. The urogenital tract inflammatory process passes in different phases; the presence of bacteria and leukocytes in semen causes oxidative imbalance, and the accumulation of pus cells leads to the initiation of phagocytosis[36]. Activation of proinflammatory cytokines modulates the prooxidative and antioxidative system, thus promoting (reactive oxygen species) burst, leading to spermatozoon peroxidative damage. Remnants of the oxidative stress process might persist in semen for a longer time after removing the infectious agent, finally resulting in spermatozoa damage[37].

The antibiotic resistance results of Gram negative bacteria revealed high resistance to amoxicillin-clavulanic acid, ceftazidime, ceftriaxone, ciprofloxacin and trimethoprim. While Gram positive resistance were presented to rifampin, erythromycin, ampicillin, doxycycline, gentamycin, trimethoprim and ciprofloxacin. Our results agree with many studies whose register same results of resistance [38,39]. Ciprofloxacin and trimethoprim were most frequently prescribed drugs to treat genitourinary tract infections caused by Gram negative bacteria[40].

Although trimethoprim has been widely used for the empirical treatment of UTI; the results of the present study showed that 52.8% and 59.7% of Gram-negative and Gram-positive isolates respectively were resistant to this drug, It is within the limits of his previous study (Taher et al., 2019)[41] was found 40-97% and 35.6-90.2% of Gram-negative and Gram-positive isolates respectively were resistant to this drug. Bacterial resistance to TMP and to sulfonamides is mediated by the following 5 main mechanisms: (1) the permeability barrier and/or efflux pumps, (2) naturally insensitive target enzymes, (3) regulational changes in the target enzymes, (4) mutational or recombinational changes in the target enzymes, and (5) acquired resistance by drug-resistant target enzymes.[42]. This study also showed high resistance rates towards cephalosporins Related to Gram negative bacteria (E.coli , Proteus spp, Enterobacter aerogenes). Proteus spp was resistant for the Ceftriaxone, Ceftazidime, Cefotaxime Attributed (66.6%), E. coli (74,4), E. aerogenes(66.5), The view was higher than the results that were given lower rates in previous studies 42.5-49.4% (Choe et al., 2018) [43]. This high rate of cephalosporins resistance that reaches to 70% resistance may due to prolonged inappropriate administration of these drugs described by doctors, and also few personal education presented by an incomplete full course of antibiotics to eradicate the pathogen to improve infection cure rates and avoid the development of any resistance or treatment failures [44]. Among our isolates are comparable to those reported by Choe et al. who also reported a very high resistance rate against fluoroquinolones among [43] Which came close to the results of the study that we obtained in terms of resistance fluoroquinolones Gram negative (62.5%) resistance rate against ciprofloxacin, and (63.3%) against levofloxacin While at Grame pozative (50%) resistance rate against ciprofloxacin, and (54.1%) against levofloxacin. These high resistance levels are likely to be driven by previous exposure to fluoroquinolones.

Conclusion:

The current study conclude dominant of E. coli, E. arogenes, S. aureus and E. faecalis as cultarabile bacteria among bacteriospermic-pyospermic patints and seem the routine antibiotics used to treat such infections like cephalosporin, trimethprime, cirpfloxacin and rifampin and erythromycin were highly resisted by bacterial siolates.

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