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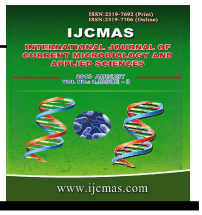


Original Research Article

Antimicrobial chemotherapy for microorganisms causing male infertility

S.Sasikumar[1]*, E.K.Madhankumar[1], D.Dakshayani[1], A.Franklin[2] and Rajkumar Samuel[3]

[1]Nova IVI fertility centre, RA.Puram, Chennai, Tamilnadu, India
[2]Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, India
[3]Hubert Enviro Care Systems (P) Ltd, Chennai, Tamil Nadu, India
***Corresponding author e-mail:** sasikumarsundararajan1@gmail.com*

Keywords

IVF; semen parameters; *Klebseilla ozaenae; Klebseilla ozaenae; Proteus vulgaris; Pseudomonas aeruginosa; Escherichia coli*

A B S T R A C T

Many studies have been performed to examine the effect of semen bacterial infection on the outcome of IVF. The presence of bacteria wanted treatment by antibiotics due to possible adverse effects on semen parameters. Complications arise when determining which bacteria are significant and which merely represent skin contamination. When examining statistics or all patients which positive semen culture. At the time of IVF , it was found no difference in the pregnancy rate when compared with patient with negative culture. It was the notion that bacteria may have an adverse effect on semen parameters and on IVF results. Staphylococcus aureus was the highest prevalent bacteria in the study. Other isolates including *Escherichia coli*, *Klebseilla ozaenae*, *Proteus vulgaris Pseudomonas aeruginosa* also recovered from the semen. These all have been reported to possess invitro spermicidal activity.Antibiotic sensitivity for the isolated organism shows their sensitivity on the antibiotics. The highly sensitive antibiotics were identified Vancomycin, Gatifloxacin, cefoperazone / Sulbactum, Amikacin, Imipenum. Minimum inhibitory concentration of thse antibiotics also determined for the better treatment to the IVF patients.

Introduction

Fertilization is sequence of events that begins with contact between spermatozoa and Oocytes leading to their fusion for embryo, which leads to the formation of a zygote and ends with the initiation of its cleavage.

A sperm cell or spermatozoa in (spermatozoa in Greek; sperm- semen and zoon-alive) is the haploid cell that is the male gamete. It is carried in fluid called semen and is capable of fertilizing and egg cell to form a zygote. A zygote can grown into a new organisms such as a human sperm cells contain half of the genetic information needed to create life. Generally the sex of offspring is determined by the sperm through, the chromosomal pair xx for female xx for female cell sperm cells were first observed by Anton van Leuvenhoek (in 1869).

Microbial contamination of culture dishes occasionally occurs in our IUF/ICSI programme, despite stringent culture conditions and the use of medium containing penicillium and streptomycin, an increasing number of infections was observed once they were routinely recorded. In study 95 cases of contaminated culture dishes were examined, I an attempt to identify possible causes, infection were observed only in IVF culture dishes and never after applying intracyloplasmic sperm injection. Identification of contaminating micro organisms should that infections were mainly caused by the Escherichia coli, Candida species of the Escherichia Coli strains isolated (73.2%) appeared to be resistant to both antibiotics used in the culture medium and 23.2% appeared to reisist either penicillin or streptomycin. The ICSI procedure must prevent colonization of the culture dishes by micro organisms. Infection in IVF culture dishes are mainly caused by bacteria starins in sensitive to the antibiotics (Kastrop *et al.*, 2007).

The word antibiotic comes from Greek anti "(against)" and bios "(life)". An antibiotic is a drug that kits or shows the growth of bacterial. These are class of antimicrobial a larger groups which also include antibacterial, antifungal, antiparasitic drugs. Antibiotics are chemical are produced of solvent from microorganisms.

Some antibiotics are bactericidal meaning that they work by killing bacteria. Other antibiotics are bacteriostatic meaning that they are work by stopping bacterial multiplication. Some antibiotics can be used to treat a wide range of infection and are known as broad spectrum antibiotics. Others are only effective against a few type of bacterial and are called narrow spectrum antibiotics. Aminoglycosides, Penicillin, Fluroquinolones, Cephalosporins, Macrolides and tetracycilne while once class is composed of multi drug each drug id unique structures.

Materials and Methods

Study materials

The semen specimen was obtained from normozooapermia donors by means of masturbation in sterile manner, and following selected uropathogenic Escherichia sp obtained from outpatients with genitourinary tract infection.

Semen collection

Semen collection of 40 patient attending to Billroth infertifility reserch center were collected in the clinic, by masturbation after a 3 days abstinence period, patient should not taken any antibiotic from one week before collecting the sample, and patients must wash their hands and genital area with soap and saline water. Samples were collected sterile plastic containers. The sample were delivered to the laboratory within less than 10 minutes and kept in the incubator adjusted to body temperature.

Spermatozoa analysis

Collected sample were subjected to the analysis of macroscopic characters of sperm.

Macroscopic observation of semen

After collection of samples, it is allowed to liquefy for 45 minutes. Color of

samples is observed. Volume of total semen ejaculation is measured pH of the semen was checked by use of the specific pH paper. These parameters were compared with the normal semen parameters to find the abnormality in sperm.

Microscopic observation of semen

Sperm count

How many sperm are present in your semen sample is important as too few can significantly your chances of conceiving. A man is considred to have a low sperm count if his sample is found to have less than 20 million sperm per ml. A diagnosis of a very low sperm count is given when sperm volume falls below 10 million per ml. Man that are found to have no sperm in their somon are said to be azoospermic. This grid is divided into 100 small boxes each 0.1 x 0.1 mm.

For undiluted sperm count all motile and non-motile sperm within 10 small boxes of this grid. Divided this number by 2. This result is the concentration of sperm in millions/ml.

$$\% \text{ Motile} = \frac{\text{motile sperm}}{\text{Motile sperm} + \text{non motile sperm}} \times 100$$

Microbiological investigation of infertility patient

Semen culture test

A routine microbiological investigation of semon was carried out for patient who comes to Billroth infertility research center, take treatment in fertility. The semen sample obtained from the infertile patient was on the above medium in sterile manner. The plates were incubated at 37°C for 24-48 hours. After incubation, the microbial colony morphology on the plates were observed recorded.

Identification characteristics

The purified bacterial cultures were identified based on gram staining, motility and other biochemical characterization.

Antibiotic sensitivity test by kirby – bauer method

Muller – Hinton agar plates were prepared aseptically. A sterile cotton swab was dipped into a well mixed saline test culture and excess inoculums were removed by pressing the saturated swab against the innner wall of the culture tube. Using the swab, the entire agar surface was streaked horizontally, vertically and around the outer edge of the plate to ensure a heavy growth over the entire surface. The selected antibiotic discs were placed in the centre of each quarter of the plate using sterile forceps. The antibiotic discs were gently pressed with the sterile forceps to ensure that the disc adhere to the surface of the agar. The plates were incubated in an inverted position for 18-24 hours at 37C. Following the incubation period, the zone of the inhibition for each antibiotic was measured either using an antibiotic zone scale on a metric scale and results were recorded.

Determination of minimum inhibitory concentration of antibiotic by broth dilution method

A stock solution is prepared by dissolving 40 mg of antibiotic in 20 ml of sterila distilled water. Sterile nutrient broth is used for bacterial organisms. Sterile 1ml broth containing tubes are used to

determine the MIC of antibiotic with stock stolution having 2 mg.ml concentration of antibiotic used. Sterile dilution in nutrient broth is done using this stock solution to get concentration from 1 mg/ml to 0.00019 mg/ml in the test tubes. In each series of the test tubes a loop full of microbial suspension is inoculated. Then all the inoculated tubes are incubated 37'C for 24 hours. Following incubation tubes are examined for the presence of microbial growth.

Result and Discussion

The patient's semen samples were analyzed and their result impressions were shown in table 1. From the semen sample urinary tract and seminal tract containing unropathogebic organisms isolated from that samples. *Staphylococcus aureus* is more frequently isolated (40%), *Escherichia coli* (25%), *klebseilla ozaenae* (15%), *Proteus vulagaris* (12.5%), *Pseudomonas aerugionosa* (10%) (Figure 1).

Staphylococcus aureus is frequently present in the semen which cause the infertility in men also majority contaminates the invitro Fertilization (IVF) procedure. Second most contamination is caused by *Eschorichia coli, Klesbeseilla ozaenae, Proteus vulgaris, Pseudomonas aeruginosa* cause the contamination as viz versa. Biochemical test results for those organisms are shown in the table 2.

For these organisms highly sensitive and moderate sensitive and resistance antibiotics are determined by antibiotic sensitive disc diffusion method. (Table 3). More specific sensitive and resistance antibiotics for each organism were shown in the table 4. From those highly sensitive antibiotics for those particular organisms minimum inhibitory concentration is determined, MIC value shown in table 5.

Many studies have been performed to examine the effect of semen bacterial infection on the outcome of IVF.The presence of bacteria wanted treatment by antibiotics due to possible adverse effects on semen parameters. Complications arise when determining which bacteria are significant and which merely represent skin contamination. When examining statistics or all patients which positive semen culture.At the time of IVF , we found no difference in the pregnancy rate when compared with patient with negative culture. This question the notion that bacteria may have an adverse effect on semen parameters and on IVF results.

Escherichia coli and *staphylococcus aureus, klebeseilla* may have an adverse effect and that patients are recommended are antibiotic chemotherapy with their specific anitbiotics like vancomycin, Gatifloxacin, Cefoperozone / sulbactum. Staphylococcus aureus was the highest prevalent bacteria in the study. Other isolates including *Escherichia coli, klebseilla ozaenae, Proteus vulgaris Pseudomonas aureginosa* also recovered from the semen. These all have been reported to possess invitro spermicidal activity.

However it has been stated that the presence of bacteria in semen may affect fertility in several ways including damaging of spermatozoa, hampering their motility altering the chemical composition of seminal fluid, this study therefore advocates for the adaption of pre-enriched solid media for optimal isolation of bacteria from semen

Table.1 Sperm analysis

S.No.	Sample	Colour	Volume	pH	Viscosity	Liquefaction	Count	Motility	WBC	Impression
1.	Patient 1	Gray Opalacent	3.7	7.8	Thin	Normal	5	40	0.2	Oligo Asthenozoospermia
2.	Patient 2	Gray Opalacent	0.5	7.6	Thin	Normal	43	22	0.5	Asthenozoospermia
3.	Patient 3	Gray Opalacent	3	8	Thin	Normal	105	57	0.8	Asthenozoospermia
4.	Patient 4	Gray Opalacent	7	7.8	Thin	Normal	7	21	0.4	Olingo Asthenozoospermia
5.	Patient 5	Gray Opalacent	2.5	7.6	Thin	Normal	33	57	0.5	Asthenozoospermia
6.	Patient 6	Gray Opalacent	2.5	8	Thin	Normal	75	47	0.6	Asthenozoospermia
7.	Patient 7	Gray Opalacent	3.5	7.5	Thin	Normal	65	58	0.2	Normozoospermia
8.	Patient 8	Gray Opalacent	1	8	Thin	Normal	18	66	0.3	Asthenozoospermia
9.	Patient 9	Gray Opalacent	3	7.5	Visous	Normal	3	56	0.9	Oligo Asthenozoospermia
10.	Patient 10	Gray Opalacent	1.5	7.9	Thin	Normal	73	36	0.8	Asthenozoospermia
11.	Patient 11	Gray Opalacent	3.5	8	Thin	Normal	47	36	0.6	Asthenozoospermia
12.	Patient 12	Gray Opalacent	1.5	7.6	Thin	Normal	53	57	0.4	Asthenozoospermia
13.	Patient 13	Gray Opalacent	2.0	7.8	Thin	Normal	45	50	0.6	Asthenozoospermia
14.	Patient 14	Gray Opalacent	1.5	8		Normal	11	55	0.4	Asthenozoospermia
15.	Patient 15	Gray Opalacent	2	7.2	Thin	Normal	73	70	0.3	Asthenozoospermia
16.	Patient 16	Gray Opalacent	3.5	8	Thin	Normal	7.5	47	0.3	Normozoospermia
17.	Patient 17	Gray Opalacent	3.5	7.9	Thin	Normal	54	63	0.5	Normozoospermia
18.	Patient 18	Gray Opalacent	5	7.5	Thin	Normal	73	73	0.6	Normozoospermia
19.	Patient 19	Gray Opalacent	2.5	7.5	Thin	Normal	3	33	0.4	Oligo asthenozoospermia

20.	Patient 20	Gray Opalacent	2.5	8	Thin	Normal	122	57	0.2	Normozoospermia
21.	Patient 21	Gray Opalacent	2.5	7.4	Thin	Normal	34	47	0.5	Asthenozoospermia
22.	Patient 22	Gray Opalacent	3.5	7.9	Thin	Normal	15	67	0.4	Asthenozoospermia
23.	Patient 23	Gray Opalacent	2.3	8	Thin	Normal	32	53	0.4	Asthenozoospermia
24.	Patient 24	Gray Opalacent	3	7.8	Thin	Normal	62	58	0.6	Normozoospermia
25.	Patient 25	Gray Opalacent	4.5	7.6	Thin	Normal	31	61	0.4	Asthenozoospermia
26.	Patient 26	Gray Opalacent	2	8	Thin	Normal	9	77	0.3	Oligo Asthenozoospermia
27.	Patient 27	Gray Opalacent	3.3	7.5	Thin	Normal	144	87	0.7	Asthenozoospermia
28.	Patient 28	Gray Opalacent	5	7.8	Thin	Normal	50	40	0.5	Normozoospermia
29.	Patient 29	Gray Opalacent	6	7.4	Thin	Normal	20	45	0.4	Asthenozoospermia
30.	Patient 30	Gray Opalacent	4.5	8	Thin	Normal	7	71	0.7	Oligo Asthenozoospermia
31.	Patient 31	Gray Opalacent	3.7	7.3	Thin	Normal	83	82	0.5	Asthenozoospermia
32.	Patient 32	Gray Opalacent	2.3	7.4	Thin	Normal	98	54	0.4	Asthenozoospermia
33.	Patient 33	Gray Opalacent	2.5	8	Thin	Normal	79	61	0.5	Asthenozoospermia
34.	Patient 34	Gray Opalacent	3	7.6	Thin	Normal	100	35	0.4	Asthenozoospermia
35.	Patient 35	Gray Opalacent	4.7	8	Thin	Normal	13	85	0.4	Normozoospermia
36.	Patient 36	Gray Opalacent	1.5	7.5	Thin	Normal	50	62	0.3	Astheno zoospermia
37.	Patient 37	Gray Opalacent	2.5	7.6	Thin	Normal	82	74	0.4	Normozoospermia
38.	Patient 38	Gray Opalacent	4	7.9	Thin	Normal	27	86	0.7	Asthenozoospermia
39.	Patient 39	Gray Opalacent	1	7.5	Thin	Normal	91	62	0.5	Asthenozoospermia
40.	Patient 40	Gray Opalacent	2.5	7.5	Thin	Normal	37	80	0.4	Normozoospermia

Table.3 Antibiotic sensitivity test

S. no.	Sample	Age	Sensitivity test			
			Organism isolated	Highly sensitive	Moderate sensitive	Resistance
1.	PATIENT 1	37	*Klebsiella ozaenae*	Cf,l,Cps, Mr,Le	Ce,Cpm	Ca,Co,Cfx
2.	PATIENT 2	29	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
3.	PATIENT 3	30	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
4.	PATIENT 4	33	*Pseudomonas aeruginosa*	I,Cps,Ak,Cf	Le,Gf,	TGC,Cfx
5.	PATIENT 5	35	*Klebsiella ozaenae*	Cf,l,Cps, Mr,Le	Ce,Cpm	Ca,Co,Cfx
6.	PATIENT 6	41	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
7.	PATIENT 7	45	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
8.	PATIENT 8	34	*Proteus vulgaris*	Ak,Cf,Le,I, Mr	Cpm,Cps	Co,Cfx
9.	PATIENT 9	44	*Pseudomonas aeruginosa*	I,Cps,Ak,Cf	Le,Gf,	TGC,Cfx
10.	PATIENT 10	38	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
11.	PATIENT 11	35	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
12.	PATIENT 12	35	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
13.	PATIENT 13	36	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
14.	PATIENT 14	38	*Klebsiella ozaenae*	Cf,l,Cps, Mr,Le	Ce,Cpm	Ca,Co,Cfx
15.	PATIENT 15	31	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
16.	PATIENT 16	33	*Klebsiella ozaenae*	Cf,l,Cps, Mr,Le	Ce,Cpm	Ca,Co,Cfx
17.	PATIENT 17	38	*Proteus vulgaris*	Ak,Cf,Le,I, Mr	Cpm,Cps	Co,Cfx
18.	PATIENT 18	42	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
19.	PATIENT 19	35	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
20.	PATIENT 10	39	*Klebsiella ozaenae*	Cf,l,Cps, Mr,Le	Ce,Cpm	Ca,Co,Cfx

21.	PATIENT 21	30	*Proteus vulgaris*	Ak,Cf,Le,I, Mr	Cpm,Cps	Co,Cfx
22.	PATIENT 22	32	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
23.	PATIENT 23	46	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
24.	PATIENT 24	40	*Klebsiella ozaenae*	Cf,l,Cps, Mr,Le	Ce,Cpm	Ca,Co,Cfx
25.	PATIENT 25	35	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
26.	PATIENT 26	29	*Proteus vulgaris*	Ak,Cf,Le,I, Mr	Cpm,Cps	Co,Cfx
27.	PATIENT 27	32	*Pseudomonas aeruginosa*	I,Cps,Ak,Cf	Le,Gf,	TGC,Cfx
28.	PATIENT 28	34	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
29.	PATIENT 29	33	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
30.	PATIENT 30	40	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
31.	PATIENT 31	28	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
32.	PATIENT 32	40	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
33.	PATIENT 33	32	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
34.	PATIENT 34	45	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
35.	PATIENT 35	36	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
36	PATIENT 36	34	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
37.	PATIENT 37	32	*Pseudomonas aeruginosa*	I,Cps,Ak,Cf	Le,Gf,	TGC,Cfx
38.	PATIENT 38	31	*Staphylococcus aureus*	Va,Le,Mr, Cf,Lz	Cps,Am,At	Ox,CA,Co
39.	PATIENT 39	37	*Escherichia coli*	CPs,Le,I, Mr,TGC	G,Pt,Cf	Cpm,Of,Am
40.	PATIENT 40	33	*Proteus vulgaris*	Ak,Cf,Le,I, Mr	Cpm,Cps	Co,Cfx

Pt - Popracillno/Tazobactum, Cps - Cofaperazone Suibactum,
Le - Levofloxacin Tb - Tobramycin
Of - Ofloxacin Mr - Meropenem
Co - Co Trimoxazole Cfx - Cefixime
G - Gentamicin, Ak - Amikacin
Va - Vancomycin Lz - Linezolid

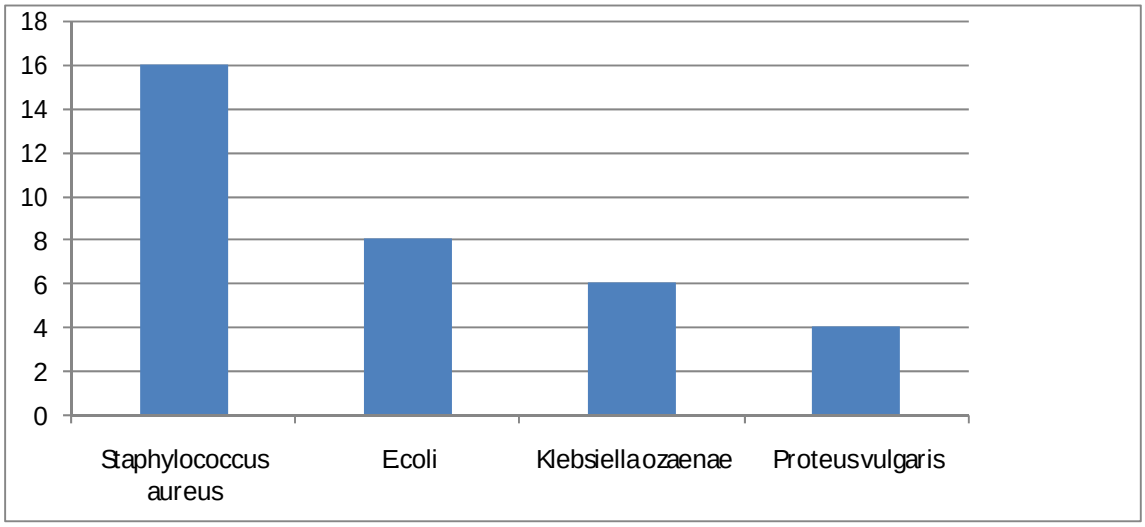
Table.4 Highly sensitive antibiotics to Bacteria

S.No.	Microorganisms	Highly Sensitive	Moderate Sensitive	Resistance
1.	*Staphylococcus aureus*	Va,Cps,Mr,Gf,Lz	Le,Am,At	Ox,Ca,Co
2.	*Escherichia coli*	CPs,Le,I,Mr,TGC	G,Pt,Cf	Cpm,Of,Am
3.	*Kilebseilla ozaenae*	Cf,Cps,Mr,Le,G	Ce,Cpm	Ca,Co,Cfx
4.	*Proteus vulgaris*	Ak,Cf,Le,I,Tb	Cpm,Cps, Mr	Co,Cfx
5.	*Pseudomonas aeruginosa*	I,Cps,Ak,Cf	Le,Gf,	TGC,Cfx

Table.5 MIC for the most sensitive antibiotics

Microorganisms	Antibiotics	MIC μg/ml
Staphylococcus aureus	Vancomycin	0.125 μg/ml
Escherichia coli	Cophapharozone sulfactum	0.31 μg/ml
Kilebseilla ozaenae	Cetiflaxacin	0.625 μg/ml
Proteus vulgaris	Amikacin	0.0156 μg/ml
Pseudomonas aeruginosa	Imipenum	0.0039 μg/ml

Figure.1 Organisms Isolated from semen sample



Antibiotic sensitivity for the isolated organism shows their sensitivity on the antibiotics. Here the generations of antibiotic presence but we use only third generation recent antibiotics for sensitivity tests, Minimum inhibitory concentration also determines for those antibiotics for better treatment of the patients so the MIC value also important to the specific antibiotics for the particular organisms.

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