The effect of Antibacterial Activity of *Streptomyces avermilitis*, SK60-8, against pathogenic bacteria isolates from follicular fluidof Inferitility Egyptian Women

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Abstract: In previous study reported bacteria in human follicular fluid. The objective of this study was to test human follicular fluid for the presence of bacteria and in vitro treatment with antibacterial agent isolated from some Actinomycetes. In this study,200 follicular fluids and vaginal swabs were collected from women undergoing Intracytoplasmic Sperm Injection (ICSI) cycles, with various causes for infertility, attending the fertility clinic at International Islamic Center for Population Studies and Research (IICPSR), Al-Azhar University, Cairo, Egypt. Bacteria isolated from follicular fluids were classified as: (1) 'Colonizers' if bacteria was detected within the follicular fluid, but not within the vaginal swab (at the time of Oocyte retrieval); or (2) 'Contaminants' if bacteria detected in the vagina at the time of Oocyte retrieval were also detected within the follicular fluid. A variety of eleven pathogenic bacterial species were isolated; *Escherichia coli* ES-1, *Staphylococcus aureus* ES-2, *Propionibacterium* ES-3, *Lactobacillusacidophilus* ES-4, *Lactobacillus plantarum* ES-5, *Lactobacillusruminis* ES-6, *Lactobacillusparacasei* ES-7, *Streptococcus agalactiae* ES-8, *Enterococcus faecalis* ES-9, *Enterococcushirae* ES-10 & *Proteus mirabilis* ES-11. The obtained bacterial species were subjected for antibacterial activity of different Actinomycete cultures isolated from different localities of Egypt, it was found that an Actinomycete culture SK60-8isolated from Soil sample collected from Kilo 60, Suez governorate, Egypt to be active against the isolated bacterial pathogens. Identification of this isolate was performed according to spore morphology and cell wall chemo-type, which suggested that this strain is a *Streptomycete*. Further cultural, physiological characteristics and phylogenetic analysis of 16S rRNA gene indicated that this strain is identical to *Streptomyces avermilitis* and then designated *Streptomyces avermilitis*, SK60-8. In its culture supernatant, this organism could produce one major bioactive compound belonging to B-Lactam antibiotics group exhibited strong antibacterial activity against the isolated bacteria-pathogens.

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1. Introduction

The presence of opportunistic pathogens in the lower female reproductive tract has been associated with adverse pregnancy outcomes after both natural and ICSI conceptions **(McClure and Goldenberg, 2009)**. Andthis study also demonstrated differences in women with colonized and contaminated follicular fluid **(Pelzer *et al.*, 2011).**

In addition, some studies have confirmed that microorganisms frequently and transiently colonize the female upper genital tract in the absence of a symptomatic infection **(Viniker,1999)**.

Studies investigating microorganisms and human follicular fluid have been mainly undertaken in women participating in ICSI cycles because of the nature of the procedures required to obtain this specimen **(Gurgan, 1993)**.

The effect of microorganisms from the ICSI culture system as a whole by pooling the results obtained for each specimen type (follicular fluid, Oocyte retrieval needle washes, semen and culture media) and seeking associations between these results and ICSI outcomes and concluded that there were no detrimental effects.

Significant decrease in the number of Oocytes retrieved from women when microorganisms were isolated from their follicular fluid **(Cottell *et al.*, 1996)**.

The search and discovery of novel microbes that produce new secondary metabolites can be expected to

remain significant in the race against new and emerging diseases and antibiotic resistant pathogens **(Kamjam *et al*., 2017)**.

Throughout the ages, natural products have been the most consistently successful source of useful compounds that have found many applications in the fields of medicine, pharmacy and agriculture. Microbial natural products have been the source of most antibiotics in current use for the treatment of various infectious diseases **(Tawiah *et al*., 2013)**.

Soil *Streptomyces* have higher antimicrobial activity against multidrug resistant microorganisms like *Staphylococcus aureus*, *E. coli* and many other Pathogens **(Sharma *et al*., 2011).** The present study was conducted to screen the ability of different Actinomycete isolates for the production of antibacterial compounds effective in inhibiting growth of the isolated bacterial human follicular fluid pathogens complaining infertility ofwomen attending the fertility clinic at International Islamic Center for Population Studies and Research (IICPSR), Al-Azhar University, Cairo, Egypt.

2. Subjects and Methods

2.1. Isolation and identification of bacterial isolates from female follicular fluid

In this study a total of 200 samples, were collected from two hundred women patient's, their the age of ranges from 20 to 40 years. Attending the assisted reproductive unit at the International Islamic Center for Population Studies and Research (IICPSRC), Al-Azhar University, Cairo, Egypt from May (2015) to June (2016). They were divided to four groups:

The two hundred of female cases divided into four groups:

- **Group one: (control)** 50 cases that fertile women (male factor or unexplained infertility).
- **Group two:** endometriosis (50 cases).
- **Group three:** polycysticovary syndrome (50 cases).
- **Group four:** tubal disease (50 case).

Two types of samples were collected from each woman: follicular fluid samples from the ovary where available, and vaginal swab samples, which were cultured for the detection and identification of microbial species was carried out.

- **The follicular fluid specimens:** were aseptically transferred to a sterile culture dish to determine if there was an oocyte present. Following transfer the oocyte to specific dish, the In Vitro Feritilzation (IVF) scientists transferred the remaining follicular fluid to a sterile 15 mL Falcon tube for storage at −80°C.
- **The vaginal swabs:** were collected prior to trans-vaginal oocyte retrieval and following the preparation of the vagina with sterile water. Preparation of the vaginal wall is performed to remove cell debris and mucous, rather than microorganisms. The vaginal swabs were collected following the vaginal preparation to ensure that the only species recovered were those remaining when the needle passed through the vaginal wall at the time of follicle aspiration **(Pelzer *et al*.,2011).**

The collected samples were cultured in aseptic condition using blood agar, Chocolate agar and MacConkey agar media at 37°C for 24 hours. The grown cultures were subcultured on trypticase soy agar, sabouraud dextrose agar and nutrient agar media. The obtained isolates were phenotypically characterized by Biologidentification system (Biolog, Hayward, Calif**) Fery *et al*.,1997).**

2.2. Testing the susceptibility of the obtained isolates to the different antibiotics:

The isolated bacterial cultures; *Escherichia coli* ES-1, *Staphylococcus aureus* ES-2, Propionibacterium ES-3, *Lactobacillus acidophilus* ES-4,, *Lactobacillus plantarum* ES-5, *Lactobacillusruminis* ES-6, *Lactobacillus paracasei* ES-7, *Streptococcus agalactiae* ES-8, *Enterococcus faecalis* ES-9, *Enterococcus hirae* ES-10 & *Proteus mirabilis* ES-11. were tested with antibiotics to check for their sensitivity pattern using the antibiotic discs methods. The antibiotics used and their concentration per disc were as follows: Gentamycin (CN 10µg), Tetracycline (TE 10µg), Cloxacillin (OB 5µg), Augmentin (Aug 30µg), Amoxycillin (Amx 25µg), Chloramphenicol (C 30µg), Cotrimoxazole (SXT 25µg), Erythromycin (E 5µg), Bacitracin (B 25µg), Sulfadiazine (SD 10µg), Streptomycin (S 15µg), Nalidixic Acid (NA 30µg), Erythromycin (E 15µg) and Methicillin (ME5 10µg) by diffusion plate methods **(cooper, 1997).**

2.3. Studying the antibacterial activity of the isolated Actinomycete cultures against the obtained bacterial isolates infecting female follicular fluid

2.3.1. Isolation of Actinomycetes

The isolation and enumeration of Actinomycete colonies from different collected samples were performed using a soil dilution plate technique on starch nitrate agar medium; composed of (g/L): Soluble starch, 20.0; $NaNO_3$, 2.0; K_2HPO_4 (anhydrous), 1.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $CaCO_3.2H_2O$, 2.0; Agar, 15 at pH 7.0 **(Tadashi, 1975)**.

The obtained Actinomycete cultures were purified using dilution plate technique as described by **(Williams and Davis, 1965).**

2.3.2. Screening for antibacterial activity of the isolated Actinomycetes

Testing antibacterial activity of the isolated Actinomycete cultures were performed by diffusion plate methods (Cooper, 1972), based on the observation of inhibition zone of bacterial growth on agar media.

2.4. Taxonomic characterization of the most active Actinomycete isolate, SK60-8.

2.4.1. Conventional taxonomy

The characterization of isolate, SK60-8 followed the guidelines adopted by International *Streptomyces* Project (ISP) (Shirling and Gottlieb,1966). Micro-morphological studies were carried out using light and scanning electron microscope (JEOL JSM 5300, JEOL Technics Ltd., Japan). Color characteristics were assessed on the scale developed by Kornerup and Wanscher **(Tresner *et al*.,1961)**.

The culture characteristics were also studied in accordance with the guidelines established by the ISP **(Shirling and Gottlieb,1966)**. Diaminopimelic acid isomers in the cell-wall and whole cell sugar pattern were analyzed using the methods reported by **(Becker *et al*., 1964)**.

The physiological and biochemical characteristics; melanin pigment production, nitrate reduction, utilization of carbon **(Shirling and Gottlieb, 1966).** activities of lipase **(Elwan *et al*., 1977)**, protease **(Chapman,1952)**,α-amylase **(Cowan and Steel,1974)** and catalase **(Jones,1949)** were tested.

2.4.2. Molecular and phylogenetic identification:

Actinomycetes isolate SK60-8 was used to inoculate 50 ml of ISP-2 broth and the culture was incubated at 200 rpm and 28°C for 24 h. The total genomic DNA was extracted according to the method of **Sambrook *et al*. (1989)**. The 16S rRNA of the isolate was amplified by PCR using a GeneAMP PCR System 9700 from PE Applied Biosystems (Perkin Elmer, Ohio, USA). The following primers were used: F27, 5'-AGAGTTTGATCMTGGCTCAG-3'and R1492 5'-TACGGYTACCTTGTTACGACTT-3' using Biolegio BV software (Biolegio, Nijmegen, Netherlands) **(Edwards *et al*.,1989)**. The PCR mixture conditions were described in Awad *et al*. (2009). The PCR products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and were detected using a gel documentation system, (Alpha-Imager 2200, CA, USA). The PCR products were sequenced using gene analysis unit in genetics laboratories of The Holding Company for Biological Products and Vaccines (VACSERA), El-Dokki, Egypt. The DNA sequences were determined using an ABI PRISM 377 DNA sequencer and ABI PRISM BigDye Terminator Cycle Sequencing (Perkin Elmer, Ohio, U.S.) at a sequencing facility at Cornell University in the U.S.A. BLAST (www.ncbi.nlm.gov) was used to assess the DNA similarities. A multiple sequence alignment and molecular phylogenetic analyses were performed using Bio Edit software **(Hall, 1999).** The phylogenetic tree was constructed using the Tree View program **(Page,1996)**.

3. Results and Discussion

3.1. Isolation and identification of follicular fluid bacterial pathogens complaining of female infertility

In the present study obtained fifty six bacterial isolates were obtained from the collected clinical samples and phenotypically characterized to the species level by Biolog identification system (Biolog, Hayward, Calif.). The results of identification revealed that, The obtained isolates were represented by eleven different bacterial species, Escherichia coli (32%), *Staphylococcus aureus* (17%), *Propionibacterium* (14%), *Lactobacillus acidophilus* (7.1%), *Lactobacillus plantarum* (7.1%), *Lactobacillusruminis* (5.3%), *Lactobacillus paracasei* (3.5%), *Streptococcusagalactiae* (3.5%), *Enterococcusfaecalis* (3.5%), *Enterococcushirae* (3.5%) & Proteus mirabilis (1.7%). These studies concluded that some bacteria (*E. coli* and *Streptococcus* spp.) in porcine follicular fluid might inhibit follicle-stimulating hormone (FSH) from binding to its receptor on granulosa cells.

In the ovary, the FSH receptor is essential for follicular development and Oocyte maturation. Such inhibition would prevent the normal hormonal functioning of FSH. It is therefore plausible that the presence of microorganisms in human follicular fluid may result in inhibition of the functioning of FSH, damage to the cumulus Oocyte complex, the subsequent immune response within the follicular fluid during folliculogenesis or in the uterus at the time of implantation either by the microorganisms themselves, or the microbial products of metabolism. Identification of bacteria colonizing the follicular fluid in couples experiencing a prolonged failure to conceive may present the clinician with an opportunity to initiate antimicrobial treatment prior to the next attempt at conception **(TothA and TothAB, 2011)**.

Data of identification of the obtained bacterial isolates were recorded in table (1) and illustrated graphically in figure (1).

3.2. Testing the susceptibility of the obtained isolates to the different antibiotics

In this study eleven different bacterial species, Escherichia coli ES-1, *Staphylococcus aureus*, ES-2, *Propionibacterium* ES-3, *Lactobacillus acidophilus* ES-4, *Lactobacillus plantarum*, ES-5, *Lactobacillus ruminis* ES-6, *Lactobacillus paracasei* ES-7, *Streptococcusagalactiae* ES-8, *Enterococcus faecalis* ES-9, *Enterococcus hirae* ES-10 & *Proteus mirabilis* ES-11. were tested for their susceptibility to 20

different antibiotics by diffusion plate methods using paper disc technique.

It was found that; Ofloxacin (Ofx 20µg), Kanamycin (K 30µg), Ampicillin (AM 10µg) and Cloxacillin (OB 5µg) had a better therapeutic effect among the antibiotics tested while the other tested antibiotics exhibited activities against some of the isolated strains. **Momoh *et al.* (2011)** reported that, ofloxacin had a better therapeutic effect among the antibiotics tested. Less than 50% of all the isolates showed resistance to ofloxacin, whereas, greater than 50% of all isolated strains showed resistance to tetracycline. Results of susceptibility of the obtained isolates to the different antibiotics are recorded in table (2).

Table (1): The obtained bacterial isolates in relation to their types, numbers and percent.

No.	Microorganism	Number	%
1	*Escherichia coli Es-1*	18	32
2	*Staphylococcus aureus Es-2*	10	17
3	*Propionibacterium Es-3*	8	14
4	*lactobacillus acidophilus Es-4*	4	7.1
5	*lactobacillus plantarum Es-5*	4	7.1
6	*lactobacillus ruminis Es-6*	3	5.3
7	*lactobacillus paracasei Es-7*	2	3.5
8	*Streptococcus agalactiae Es-8*	2	3.5
9	*Enterococcus faecalis Es-9*	2	3.5
10	*Enterococcus hirae Es-10*	2	3.5
11	*Proteus mirabilis Es-11*	1	1.7
Total		56	100%

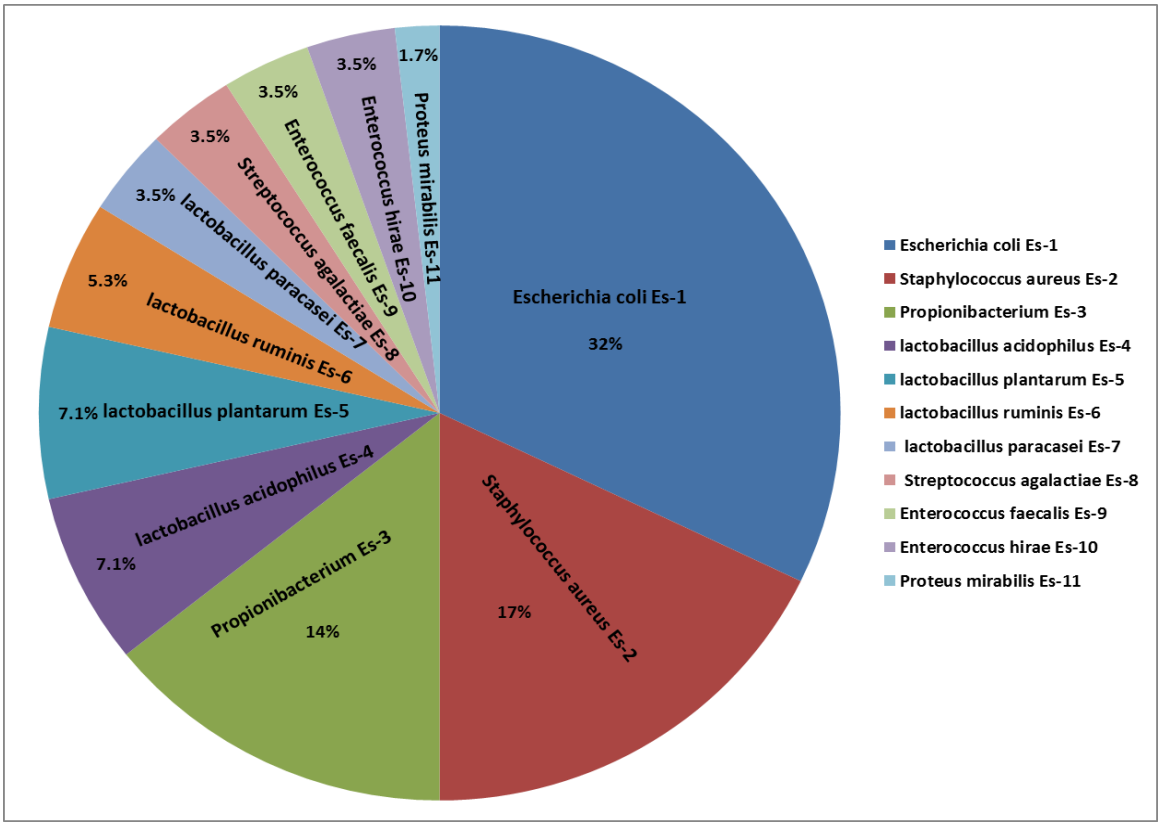


Figure (1): Percentages of the obtained bacterial isolates.

Table (2): Susceptibility of the obtained isolates to the different antibiotics using paper disc diffusion method

No.	Antibiotics	Mean diameter of inhibition zone (mm) of the tested bacterial strains										
		E. Coli ES-1	***S.aureus ES-2***	***Propionibacterium ES-3***	***L.acidophilus ES-4***	***L.plantarum ES-5***	***L.ruminis ES-6***	***L.paracasei ES-7***	***S.agalactiae ES-8***	***E.faecalis ES-9***	***E.hirae ES-10***	***P.mirabilis ES-11***
1	**Gentamycin (CN 10µg)**	0	20	16	18	15	0	0	13	12	20	0
2	**Tetracycline (TE 10µg)**	22.5	23	18	24.5	16.9	0	20	19	17	10	3
3	**Cloxacillin (OB 5µg)**	25	23	26	27	24	21	22	23	26	15	14
4	**Augmentin (Aug 30µg)**	18	17	23	15	0	0	0	15	17	14	12
5	**Amoxycillin (Amx 25µg)**	3	0	22	18	26	27	10	12	15	17	14
6	**Chloramphenicol (C 30µg)**	20	23.5	20	23	0	0	15	12	10	14	8
7	**Cotrimoxazole (SXT 25µg)**	0	0	0	0	0	0	0	0	0	0	0
8	**Erythromycin (E 5µg)**	16	15	20	0	24	22	13	12	10	14	8
9	**Bacitracin (B 25µg)**	17	16	18	15	22	19	20	13	12	17	3
10	**Sulfadiazine (SD 10µg)**	0	0	0	0	0	0	0	0	0	0	0
11	Streptomycin (S 15µg)	6	19	16	18	15	0	12	0	14	13	5
12	Nalidixic Acid (NA 30µg	20.5	23	20	18.5	16.9	0	16	15	13	10	9
13	Erythromycin (E15µg	24	21	26	27	24	21	20	22	12	15	13
14	Methicillin (ME5 10µg)	12	15	23.5	21	16	20	12	14	18	16	6
15	Ampicillin (AM 10µg)	0	0	14	21	16	20	12	14	18	16	6
16	Ciprofloxacin (CIP 10µg)	23	20.5	14	23	0	0	10	0	0	14	7
17	Colistin (CL 20µg)	0	0	0	0	0	0	0	0	0	0	0
18	Kanamycin (K 30µg)	22	24	26	23	20	22	10	17	15	20	10
19	Polymyxin B (PB 10µg)	22	19	18	15	22	19	10	12	13	10	5
20	Ofloxacin (Ofx 20 µg)	26	28	27	30	25	23	21	25	28	22	16

3.3. Studying antibacterial activity of the isolated Actinomycetes against follicular fluid-bacterial pathogens

One hundred Actinomycete isolates were isolated from different soil and water samples that collected from different localities in Egypt, these isolates were screened for their antibacterial activity against the isolated follicular fluid bacterial pathogens. It found that, sixty isolates (60%) exhibited various degrees of activities against all tested bacteria, on the other hand, forty isolates (40%) failed to exhibit antibacterial activity against the all tested bacteria.

The results were recorded in table (3) revealed that, Actinomycete isolates;

SK60-8, SK60-10, SK60-15, SK60-30, SK60-46, Mar. M-51, Mar. M-60, Mar. M-71, Al. Ak.–81 and Al. Ak.–91 exhibited the highest antibacterial the selected as the highest antibacterial producing isolates and SK60-8 was most active one, thus it was selected for further studies concerning its identification. **Oskay *et al*. (2009)** reported that, screened 50 Actinomycetes

isolated from soil against several human pathogens. It found that 34% of strains produced antibiotics.

Table (3): The selected actinomycete isolates with the highest antibacterial activity.

Isolate No.	Mean diameter of inhibition zone (mm) of the tested microbial strains										
	E. Coli	S.aureus	Propionibacterium	L.acidophilus	L.plantarum	L.ruminis	L.paracasei	S.agalactiae	E.faecalis	E.hirae	P.mirabilis
	ES-1	ES-2	ES-3	ES-4	ES-5	ES-6	ES-7	ES-8	ES-9	ES-10	ES-11
Sk60-8	28	30	26	20	25	22	27	21	23	22	19
Sk60-10	20	18	22	18.5	20	17	22	15.5	18	15	16.5
Sk60-15	19	17	13	20	22	15	13.5	11	10.5	8.5	9
Sk60-30	20	22	19	17	15	14.5	15	12.5	16	17.5	13
Sk60-46	19	17	20	15	20	14	12	16	18	15	13
-Mar.M 51	19	22	16	14.5	10	11.5	8.5	12	9.5	15	13
-Mar.M 60	17	16	14	10.5	9.5	9.5	6.5	10	8	12	10
-Mar.M 71	14	12.5	10.5	15	10	9.5	11	9	6.5	8.5	6.5
Al.Ak-81	16	18	15.5	13.5	10.5	11	7.5	12	8.5	6.5	5
Al.Ak-98	10	18	16	15	13	12	10	8.5	7	5.5	6

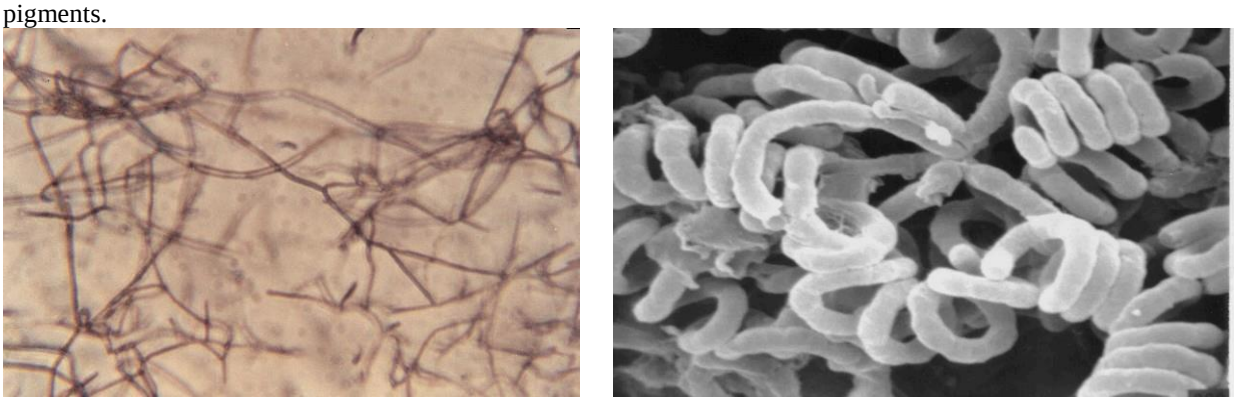
3.4. Taxonomic characterization of Actinomyceteisolate, SK60-8

3.4.1. Conventional Taxonomy

Micro-morphological studies of Actinomycete isolate, SK60-8 was studied through light microscopy (x400) and scanning electron microscope (x4500), results revealed that, the spore chains are rectiflexibles with oval spore shape figure (2a, b).

The cultural characteristics of Actinomycete isolate, SK60-8grown on different ISP media (table 4) showed that, the aerial hyphae of the strain was greenish grey. In the culture media tested, the strain was found to produce organish brown diffused pigments.

The entire hydrolysate cell of this strain contained LL-diaminopimelic acid (LL-DAP) and glycine indicating that, the strain has a chemo-type I cell wall but no characteristic sugars could be detected. Cell-wall composition analysis is one of the main methods that can be employed to identify the chemotaxonomic characteristics of *Streptomyces*; the presence of LL-DAP in the cell wall also signifies that this strain is *Streptomyces* **(Lechevalier *et al.*, 1970).** The physiological and biochemical properties; were included Carbon sources utilization; Tolerance to NaCl; Growth pH; Growth temperature and growth in table 5.



A **B**

Figure (2): A; Phase-contrast micrograph of Actinomyce tesk 60-8 showing rectiflexible shaped mycelium (x600). B; Scanning Electron Microscopy (SEM) showing rectiflexible mycelium with oval spore (x4500).

Table (4): Cultural characteristics of Actinomycete isolate SK60-8 on different ISP-media.

Medium	Growth	Substrate mycelium	Aerial mycelium	Diffusible pigments
Tryptone yeast extract broth (ISP–1)	Good	brown	Greenish grey	Organish brown
Yeast -malt extract agar (ISP–2)	Good	Organish brown	Greenish grey	Organish brown
Oat meal agar (ISP–3)	Good	Organish brown	Greenish grey	None
Inorganic-trace salt- starch agar (ISP–4)	Good	Organish brown	Greenish grey	None
Glycerol asparagine agar (ISP–5)	Good	Organish brown	Greenish grey	Organish brown
Peptone yeast extract iron agar (ISP–6)	Good	Organish brown	None	None
Tyrosine agar (ISP–7)	Good	Organish brown	Greenish grey	Organish brown

Table (5): Morphological, physiological and biochemical characteristics of Actinomycete isolate SK60-8

Character	Results	Character	Results
Melanin pigment:			
Peptone-yeast extract iron agar	-[b]	**Tolerance to NaCl (%)**	
Tyrosine agar	+	4.0	++
Tryptone-yeast extract broth	-	5.0	+++
Hydrolysis of:		6.0	–
Protein	+[c]		
Urea test	-	**Growth temperature ºC**	
Utilisation of C-sources		10	–
D-Glucose	+	25 30	+ +++
D (+) Trehalose	+		
D-Fructose	+	**Growth pH**	
Sucrose	+	7	+++
Maltose	+	8	+
Raffinose	+	9	–
D-Mannose	-		
L (+) Arabinose	-		

[b] (-) = negative, [c] (+) = moderate, [d] (+++) = abundant, [e] (++) = good growth,

3.4.2. 16S rRNAgene sequencing and phylogenetic analysis

To confirm the identification of the isolate SK60-8, 16S rRNA gene sequence of this isolate was compared to sequences of 10 *Streptomyces* spp. through multiple sequence alignment. The primer pair, F27/R1492 was used to amplify the fragments of the genomic DNA's expected size (1500 bp), this primer pair was especially used to amplify the 27-bp and 1492-bp fragments. Experimental analysis of the PCR amplification was studied through agarose gel electrophoresis figure (3). The results obtained were in agreement with those of **Edwards *et al.* (1989)** who found that these primers were specific for bacteria. **Hongyu *et al*. (2011)** isolated and identified soil *Streptomyces* sp.. A phylogenetic tree was derived from the distance matrices using a neighbor-joining method figure (4). A good congruence was found between the 16S rRNA sequence of *Streptomyces avermilitis* BA000030.4and of local isolate, sk60-8.

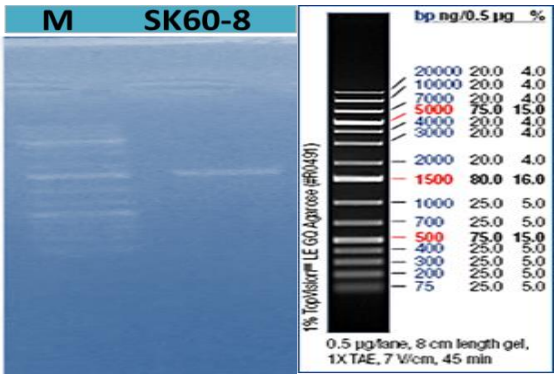


Figure (3): Amplified fragment of 16S rRNA gene, (M): Marker DNA

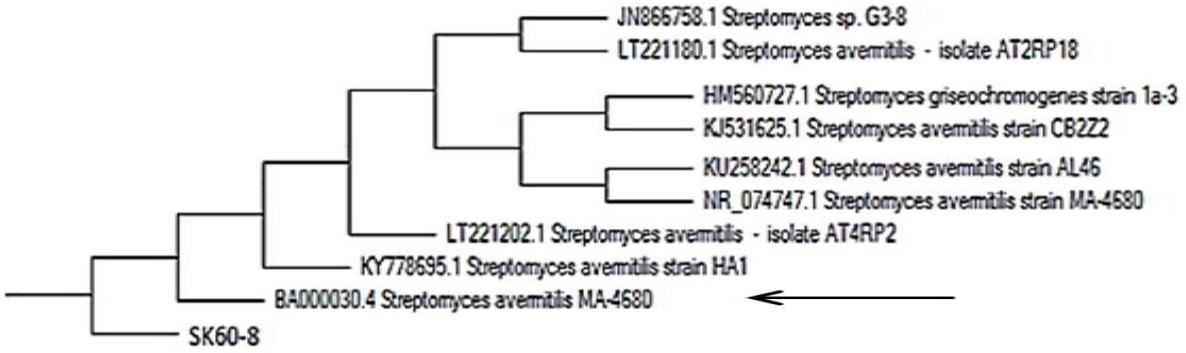


Figure (4): Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequence of isolate, SK60-8 and phylogenetically related member of this genus.

The morphological, physiological and phylogenetic characteristics of Actinomycete isolate, SK60-8 suggested that, this isolate have high similarity with reference strain *Streptomyces avermilitis* **(Omura,2001).** The comparative study of identification properties of the two strains are recorded in table (6).

At the turn of the century, a shift driven by new technologies were started in search of exploitable biology. This shift is exemplified by the extent of biodiversity now revealed and recognized by biologically informative and data-rich methods functioning at the molecular scale. Such methods are often employed for characterizing organisms and defining taxon-property relationships through high-throughput screening and the PCR and DNA sequencing **(Alan *et al*.,2000).** In this study, the phylogenetic analysis coupled with a conventional method related to SK60-8 indicated that the most closely-related strain is *Streptomyces avermilitis* DSM (Accession number BA000030.4) therefore, *Streptomyces avermilitiss*, SK60-8 is proposed as its name. The use of genotypic and phenotypic techniques gave a better resolution in species-level identification **(Mizui *et al*.,2004).**

Table 6: A comparative study of identification properties of the isolate, SK60-8 in relation to the reference strain *Streptomyces avermilitis* (Omura,2001)

Characteristics	Local isolate SK60-8	*Streptomyces avermilitis*
1- Morphological characteristics		
Spore mass	brown	brown
Spore chain	Rectiflexibles	Rectiflexibles
Spore surface	Smooth	Smooth
Diffusible pigment	+	+
2- Chemotaxonomic characteristics		
DAP	LL-DAP	LL-DAP
Sugar pattern	–	–
3- Physiological characteristics		
A-Utilization of carbon sources		
D-Glucose	+	+
D-Fructose	+	+
Sucrose	+	-
L-Rhamnose	(–)	-
Meso-Inositol	-	-
C- Growth at 45 °C	-	-
D- Growth at (% w/v)		
NaCl (5.0%)	+	+

(+) = moderate, [c] (-) = negative, (+++) = abundant

3.5. Antibacterial activity of *Streptomyces avermilitis,* SK60-8.

The Actinomycete *Streptomyces avermilitis*, SK60-8 was allowed to grow on fermentation nutrient medium under optimum environmental and nutritional conditions. In its culture supernatant, this organism could produce one major compound strongly inhibits the growth of the isolated follicular fluidbacterial pathogens complaining of female infertility. The isolated compound was found to be belonging to β-lactam group **(Macdougall., 2011)** as a derivative of monobactams which was isolated from *Streptomyces avermiliitis* **(Dalhoff *et al*.,2006).**

3.3. Comparing between the most potent antibiotics exhibit different activities and antibiotic Monobactams SK60-8 against bacterial isolates complaining of female human follicular fluid. It was found that the produced antibiotic Monobactams SK60-8 has antibacterial activity with MIC lower than the tested antibiotics.

Table (7): Comparing between standard antibiotics and produced antibiotic Monobactams SK60-8.

Antibiotic	Mean diameter of inhibition zone (mm) of the tested bacterial strains										
	ES1 E-coli	***ES2 Staphylococcus aureus***	***ES3 Propioni Bacterium SSP***	***ES4 Lacto bacillus acidophilus***	***ES5 Lacto bacillus plantarum***	***ES6 Lacto bacillus ruminis***	***ES7 Lacto bacillus paracasei***	***ES8 Strepto coccus agalactiae***	***ES9 Entero coccus faceli***	***ES10 Entero coccus hiare***	***ES11 Proteus mirabilis***
Cloxacillin (OB 5µg)	**27**	**25**	**22**	**20**	**24**	**21**	**22**	**23**	**26**	**15**	**14**
Kanamycin (K 30µg)	**24**	**26**	**22**	**23**	**20**	**22**	**10**	**17**	**15**	**20**	**10**
Ofloxacin Ofx (20 µg)	**28**	**29**	**25**	**23**	**25**	**23**	**21**	**25**	**18**	**22**	**16**
Monobactams (4µg) SK60-8	**28**	**30**	**26**	**20**	**25**	**22**	**25**	**21**	**23**	**22**	**19**

4. Conclusion

The presence of bacteria in follicular fluid may be a significant contributor to adverse ICSI outcomes. Treatment with antibacterial may increase ICSI treatment success rates. In conclusion, it is believed that a rich source of new drug candidates can be potentially obtained from soil organisms or their metabolites. This preliminary screening of soil Actinomycetes for new antibacterial antibiotic revealed their potential to yield potent bioactive compounds for drug discovery programs.

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6. Ethics statement

Ethical approval was obtained from the International Islamic Center for Population Studies and Research (IICPSRC), Azhar University, Cairo, Egypt. All patients provided informed written consent for their follicular fluids to be used in this study and gave permission for researchers to access medical records to obtain their reproductive history.

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