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Double Inhibitory Effect of Extracellular Protein of Marine *Streptomyces tendae* against Different Strains of MRSA

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ABSTRACT

There twenty nosocomial strains of methicillin-resistant Staphylococcus aureus (MRSA) were collected over Great Cairo during three months. Some facts were observed and recorded during that survey; the male infection percent (65%) was larger than of female (35%), the percent of infected blood cases was more than the others (45%) followed by diabetic foot (25%), vagina and pubic (10%), and sputum and buccal cavity (5%). On the other hand, marine soils were professionally collected from different coastal locations in Egypt. Forty marine actinomycete isolates were isolated and screened to produce β-lactamase inhibition activity. The screening test resulted in presence of only five isolates produced β-lactamase inhibition activity included a most potent one that symbolized Sm4. The latter was identified as Streptomyces tendae by using both classical and molecular techniques. The purification of β-lactamase inhibitory protein was carried out by using ion exchange and gel filtration column chromatographs, and then separated at 40 KDa by using SDS-PAGE. Simultaneously, purified β-lactamase inhibitory protein has produced antibacterial activity, so it has double inhibitory effect against MRSA strains. The β-lactamase inhibitory protein was found composed of 17 amino acids; however, therionine has a highest content (90 moles %) followed by arginine (75 moles %) and alanine (70 moles %).

Keywords: Beta-lactams, Beta-lactamases, MRSA, Antibacterial proteins, Protein purification.

INTRODUCTION

Beta-lactams are broad spectrum antibacterial antibiotics, which significantly inhibit the growth of Gram-positive and Gram-negative bacteria.Beta-lactam antibiotics include penicillins, cephalosporins, monobactams, carbapenems, cephamycins and oxacephems, whatever in natural or semisynthetic forms [1].Although β-lactam ring is a main nucleus forβ-lactam antibiotics, the mother nucleus of penicillins and cephalosporins is 6-aminopenicillanic acid and 7-aminocephalosporanic acid respectively (Figure 1).Beta-lactams inhibit the biosynthesis of peptidoglycan layer by combining and inactivating transpeptidase enzyme. Although β-lactams are effective antibiotics and widely used in the treatment of bacterial infectious diseases, bacterial resistance especially by*Staphylococcus aureus* had been monitored, especially in the developing countries. There are various mechanisms for β-lactams resistance; however, the most common one is inactivating enzymes called (β-lactamases),which split the β-lactam ring [2]. On the other hand, there are specific proteins called penicillin-binding proteins (PBPs), which inactivateβ-lactam ringby forming serine ester bonds unlike that ofβ-lactamases[3, 4, 5].

Beta-lactamases were classified according to various factors, such as hydrolytic spectrum, susceptibility to inhibitors, and whether they are encoded by the chromosome or by plasmids [6, 7,4,8,9, 10]. However, the more advanced classification of β-lactamases was organized [8, 4]. Where, β-lactamases had been classified according to their favorite substrate among penicillin, oxacillin, carbenicillin, cephaloridine, expanded-spectrum cephalosporins, and imipenem and also according to their susceptibility to inhibition by clavulanate. Moreover, β-lactamases were

classified into four classes according to their sequence; A, B, C, and D. Although classes A, C, and D utilize the serine ester mechanism, class B use zinc ions to hydrolyze the β-lactam ring [11, 12].Although all phenotypic classifications that mentioned above have reasonable organization, they face a great problem called mutations, which greatly alter the substrate specificity [13, 14] and inhibitor susceptibility [15], and thereby change the group to which an enzyme is assigned. Although plasmid-mediated β-lactamases are distinct from the chromosomal types, few overlaps are existing. For example, SHV-1 β-lactamase is a plasmidic type [16, 17], nevertheless, it is well known as chromosomal type of *Klebsiella pneumoniae*.

Interestingly, β-lactamase inhibitory proteins (BLIPs), including BLIP-I and BLIP-II are constitutively produced from the soil *Streptomyces* bacteria[18, 19]. The new active pharmaceutical formulae usually contained β-lactam antibiotic and β-lactamase inhibitory protein to completely inhibit the growth of resistant bacteria[20, 21]. The β-lactamase inhibitors were classified into two types according to their action; reversible and irreversible. The latter is more effective than the former due to its permanent effect so it is called "suicide inhibitors" such as clavulanic acid, sulbactam and tazobactam. Notably, clavulanic acid is the most common and effective β-lactamase inhibitor, which first isolated from *Streptomyces clavuligerus*[22]. Other clavulanic acid producers include *Streptomyces jumonjinensis*, *Streptomyces katsurahamanus*, *Streptomyces* sp. FERM-P 2804 [23] and *Streptomyces* sp. NRC-35 [24, 25].

Marine actinomycetes are well known have bioactive secondary metabolites including enzyme inhibitors and antimicrobial substances. Revathy et al. [26]studied the antioxidant and enzyme inhibitory potential of *Streptomyces* sp. VITMSS05 strain, isolated from Marakkanam, southern coast of India. Although actinomycetes are well known as Gram-positive bacteria, which considered a main source of antibiotics biosynthesis, a little is known about the actinomycetes diversity of marine sediments, which is an inexhaustible resource that has not been properly exploited[27]. Actinomycetes comprise 10% of the total bacteria colonizing marine aggregates [28]. It is a boon in marine bioprospecting for the exploration and exploitation of the rich biological and chemical diversity found in marine organisms that inhabit the oceans.

In this study, there remarkable protein was isolated from the filtrate of marine *Streptomyces tendae*. This protein has unique feature, where it was found have a binary inhibitory effect against different strains of nosocomial MRSA, where it was considered β-lactamase inhibitory protein and antibacterial agent definitely against Gram-positive bacteria at the same time. *Streptomyces tendae* was isolated from marine soil collected from Al-Manzalah Lake, Kafr Al-Sheikh governorate, Egypt.

MATERIALS AND METHODS

Collection of MRSA strains

There were twenty unrepeated methicillin-resistant *Staphylococcus aureus* (MRSA) strains collected from different hospitals over Great Cairo during three months as shown in Table 1. These strains were brought to the laboratory and immediately cultured in nutrient broth medium. Incubation has been carried out at 37°C for 48 hours. Furthermore, loopful from each bacterial suspension was streaked on the surface of mannitol salt agar medium and then incubated at 37°C for 24 hours to confirm presence of pure cultures of *Staphylococcus aureus.*

Collection of marine soil samples

There ten marine soil samples were collected from different coastal locations in Egypt summarized in Table 2. The samples were brought to the laboratory in sterile polythene bags, and stored in refrigerator for further study [29].

Isolation of marine actinomycetes

Marine actinomycetes were isolated from different marine soils according to Kuster and Williams [30]and Haritha et al[31].

Determination of minimal inhibitory concentration (MIC)

The MIC for each clinical bacterial strain was determined according to Jennifer [32].

Detection of β-lactamase inhibition activity

Beta-lactamase inhibition activity was detected by using chromogenic cephalosporin spot test [33].

Screening test

Marine actinomycete isolates were screened against MRSA strains to produce β-lactamase inhibition activity using agar diffusion disc method [34].

Identification of actinomycete isolate
The most potent actinomycete isolate was identified according to Shirling and Gottlieb [35].

16S rRNA identification by PCR
Identification was carried out by 16S rRNA sequencing. 16S rRNA was amplified in a thermocycler (Perkin Elmer Cetus Model 480) by using universal primers of 27f (5' -AGA GTT TGA TCC TGG CTC AG -3') and 1525r (5'-AAG GAG GTG ATC CAG CC-3') under the following condition: 94°C for 5 min, 35 cycles of 94°C for 60 s, 55 for 60 s, 72°C for 90 s and final extension at 72°C for 5 min. The product was directly sequenced by a BigDye terminator cycle sequencing kit (PE Applied Biosystems USA) on an ABI 310 automated DNA sequencer (Applied Biosystems, USA). Homology of the 16S rRNA sequence of isolate was analyzed by using BLAST program from GenBank database [36].

Preparation of cell free extract
The starch casein broth medium (1 liter) was inoculated by most potent actinomycete isolate and then incubated at 28°C for 7 days with shaking at 180 rpm for good aeration. After incubation period, the filtrate was centrifuged at 10,000 xg for 10 minutes. The supernatant (cell free extract) which contains β-lactamase inhibitory protein was taken and subjected to precipitation by ammonium sulphate.

Precipitation by ammonium sulphate
The cell free extract containing β-lactamase inhibitory protein was gradually supplemented with a wide range of ammonium sulphate (10 to 90%) to precipitate all present proteins included target protein. These proteins were picked up in nine fractions according to their molecular weight. At each concentration of ammonium sulphate, the patch was left for 2 h at 4°C, and then followed by centrifugation at 8000 xg for 20 minutes at 4°C. The pellet was dissolved in 10 ml phosphate buffer at pH 7.5 (fraction).

Quantitative estimation of total protein content
The total protein content in each fraction as well as bacterial filtration was quantitatively estimated according to Lowery et al.[37].

Purification of β-lactamase inhibitory protein
The β-lactamase inhibitory protein was purified by using ion exchange column chromatography and gel filtration [38].

Molecular mass determination
The homogeneity and the relative molecular mass in denaturing conditions were carried out by analytical polyacrylamide gel electrophoresis (SDS-PAGE) on a 4-15% polyacrylamide gel as described by Laemmli [39], Blackshear [40], and See and Jackowski [41].

Determination of an antibacterial activity of target protein
The antibacterial activity of target protein was determined by using agar diffusion disc method[34].

Amino acid analysis
The amino acids content and sequence of β-lactamase inhibitory protein were determined by using HPLC equipment according to Cohen and Strydom[42]and Almeida et al. [43].

RESULTS AND DISCUSSION

Collection of clinical strains of MRSA
There were twenty nosocomial strains of MRSA collected during three months (from November – 2015 to January – 2016) from different hospitals over Great Cairo (Table 1). In the beginning, only six strains were collected from Sayed Galal hospital (Cairo), included five cases of male (83.3%) and one case of female (16.7%).The patient age whether male or female ranged between 22 and 55 years old. The chronic diseases are absent except in two male cases which are diabetics (33.3%). The infection site was variable; foot, blood, vagina and pubic. The strain of female case was isolated from vagina, while one strain was isolated from diabetic foot of male, two strains were isolated from two blood samples of two different male cases, and finally three strains were isolated from three pubic swabs of three different male cases.

There were five strains were collected from Om-El-Masreen hospital (Giza), included three cases of male (60%) and two cases of female (40%). The patient age whether male or female ranged between 20 and 40 years old. The chronic diseases are absent except in one female case which is diabetic (20%). The infection site was variable;

Buccal cavity, blood, and sputum. The two strains of female cases were isolated from sputum and blood, while three strains of male cases were isolated from one swab of buccal cavity and two blood samples. Furthermore, four strains were collected from Dar Alfouad hospital (6-October), included three cases of male (75%) and one case of female (25%). The patient age whether male or female ranged between 25 and 45 years old. The diabetes as a chronic disease was present in all cases (100%). The infection site was variable betweenfoot and blood; however,one strain of female case was isolated from diabetic foot, while the other three strains of male cases were isolated from one sample of blood and two swabs of diabetic foot.

Moreover, three strains were collected from Holwan Alaam hospital (Holwan), all of them are female cases (100%), whilst the male cases were absent (0%). The patient age ranged between 20 and 45 years old. The chronic diseases were found in only two cases, one of them was found have suffered from hypertension (33.3%), while the another one suffered from diabetes (33.3%). The infection site was variable between vagina and blood; however, one strain was isolated from vagina, while the other two strains were isolated from two samples of blood. Eventually, only two strains were collected from Nile hospital (Shobra Al-Khima), both of them are male cases (100%), whilst the female cases were absent (0%). The patient age was found at 40 years old. The two cases were found have suffered from diabetes as a chronic disease (100%). The infection site was variable between foot and blood; however, one strain was isolated from diabetic foot and another one was isolated from blood sample.

According to the previous survey, there are some facts should be mentioned; the males are more susceptible to MRSA infection than females, where their percentage in this study was 65% and 35% respectively. Furthermore, cases of chronic diseases, especially diabetes are slightly more susceptible to MRSA infection than others, where their percentage in this study was 55% and 45% respectively. Moreover, blood is more susceptible organ to MRSA infection (45%), followed by diabetic foot (25%), vagina (10%), pubic (10%), sputum (5%), and buccal cavity (5%). The Rhode Island Hospitals reported that, MRSA can infect bloodstream through penetration the body barriers by a germ (bacterium) in many ways, like through acatheter, or medical tube in your vein such as a “central line” that you may have when you are very sick in the hospital. Although the number of people with MRSA bloodstream infections is lower than a few years ago, these infections can be dangerous. On the other hand, diabetic foot infections (DFI)in most cases lead to gangrene and amputation. In general, diabetes is immunopathic or immunosuppressed disease, which predisposing the patient to infection, especially by Gram-positive bacteria. Certainly bacterial resistance worsens the outcome since the empiric therapy may be ineffective, delaying appropriate antibiotics. Skin and skin structure infections (SSSI) are predominately caused by Gram-positive bacteria. This, however, includes resistant Gram positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA). Approximately 30% of diabetic foot ulcers (infected and uninfected) will be colonized with MRSA. Appropriate antibiotic therapy usually involves culturing the wound and choosing an antimicrobial based on sensitivity testing. However, wound cultures are not used to diagnose an infection, just too direct therapy. This makes the initial choice of antibiotic imperative to the rapid resolution of the infection. Empiric antibiotic therapy choice is based on several factors: risk factors for MRSA, infection severity, patient allergies and concomitant medications, and inpatient/outpatient therapy.

Furthermore, vaginal-rectal carriage of MRSA has been found associated with development of postpartum fever. Although risk factors associated to colonization with MRSA strains during pregnancy have not been fully characterized, associations with race, parity, type of birth, and colonization with group B streptococci have been suggested. It is known that incidence of community-associated methicillin-resistant *Staphylococcus aureus*(CA-MRSA) infections varies among different communities and populations, and apparently, pregnant women are more susceptible and have risk factors that predispose them to developing these infections [44, 45].Nevertheless, there is a scarcity of epidemiologic reports about MRSA infections present in pregnant and puerperal women [44]. Oscar et al. [46] reported that, the vaginal infection rates between 14% and 17.1% in pregnant women have been recently reported, raising interest about the potential risk in postpartum women and in neonates from colonized mothers. MRSA has been isolated as a cause of oral infection in the UKbut these cases have been associated with oral surgery in a hospital setting [47].

Isolation and screening of marine actinomycete isolates

The marine soil samples were collected from different locations in Egypt during one month, and picked up at five meters depth from the water surface (Table 2). Constantly, there forty unrepeated marine actinomycete isolates were isolated and completely purified, and then subcultured on starch casein agar medium. These isolates were screened against twenty nosocomial strains of MRSA to exhibit β-lactamase inhibition activity. The screening test resulted in; only five isolates of marine actinomycetes have inhibited the growth of all strains of MRSA in presence of methicillin (1000 µg/ml). According to the inhibition degree, one actinomycete isolate among five was found have a highest β-lactamase inhibition activity. This most potent isolate was symbolized "Sm4".

Out of this, marine microorganisms particularly actinomycetes consider a main source of diverse effective primary and secondary metabolites, which widely use in different fields[48, 49].Claude Zobell as a pioneer of marine microbiology became active in delineating the vast numbers and diversity of true marine bacteria. One of the early isolations of secondary metabolites from marine sources was the isolation of cephalosporin in 1948 by Giuseppe Brotzu. Cephalosporin (cephalosporin C, 6) was isolated from the fungus *Cephalosporium acremonium.* Moreover, actinomycetes constitute a significant component of the microbial population in most soils and counts of over 1 million per gram are commonly obtained [50]. The soil is the most prolific source of actinomycetes, which are found to produce antibiotics and other useful metabolites. A gram of rich soil contains 10^6 streptomycetes colony-forming units and 10^4 and 10^5*Micromonospora*, as well as various other actinomycete genera. In soil, streptomycetes are finding plenty of surfaces to support their mycelial growth. The spores contribute to the survival over longer periods of drought, cold and anaerobic conditions. It appears that streptomycetes exist for extended periods as resting arthrospores that germinate in the occasional presence of exogenous nutrients. Although nutrient availability is a major factor controlling the activity of soil actinomycetes, various other environmental factors also exert an influence. Actinomycetes have been isolated from marine environment largely from sediment samples from the continental shelf or from brackish water environments such as salt marshes [51]. Goodfellow and Hynes [52] suggested that marine environment might be a valuable source for the isolation of actinomycetes with the potential to yield useful products. However, it has not yet been resolved whether actinomycetes are part of the autochthonous marine microbial community of sediments or whether actinomycetes isolated from marine sediment samples originate from terrestrial habitats and were simply carried out to sea in the form of resistant spores.

Identification of actinomycete isolate "Sm4"

The most potent marine actinomycete isolate was identified as *Streptomyces tendae* according to Shirling and Gottlieb [35]. Where, morphological, physiological and cultural characteristics were exactly determined. Notable, the electron micrograph has been illustrated that spiral spore chain was clearly observed, which consists of ellipsoidal spores that have warty surface (Figure 2). Moreover, the cultural characteristics were determined, included growth rate, color of aerial and substrate mycelia and color of diffusible pigments on different seven recommended media as mentioned in ISP (Table 3). Interestingly, on tryptone yeast extract broth, the growth rate was found poor with light gray aerial mycelia and light gray yellowish brown substrate mycelia, and the diffusible pigment has not been observed; on yeast-malt extract agar, the growth rate was found moderate with pinkish gray aerial mycelia and light orange yellow substrate mycelia, and the diffusible pigment has not been observed; on oat-meal extract agar, the growth rate was found good with light gray aerial mycelia and light yellowish brown substrate mycelia, and the light gray yellowish brown color was found diffused in lower agar layers; on inorganic salt starch agar, the growth rate was found moderate with pinkish gray aerial mycelia and light gray yellowish brown substrate mycelia, and the diffusible pigment has not been observed; on glycerol asparagine agar, the growth rate was found good with light gray aerial mycelia and light gray yellowish brown substrate mycelia, and the diffusible pigment has not been observed; on peptone yeast extract iron agar, the growth rate was found poor with pinkish gray aerial mycelia and light gray yellowish brown substrate mycelia, and the diffusible pigment has not been observed; on tyrosine agar, the growth rate was found poor with pinkish gray aerial mycelia and light gray yellowish brown substrate mycelia, and the diffusible pigment has not been observed. Furthermore, biochemical and physiological characteristics were studied (Table 4). LL-Diaminopimelic acid (LL-DAP) has been detected; however, sugar pattern was not being detected. Furthermore, Sm4 isolate was found have produced for amylase, protease, pectinase, catalase, cellulase, nitrate reductase and melanoid pigment. On the other hand, it has not been produced for lipase, lecithinase and hydrogen sulfide (H_2S). Constantly, this isolate could be hydrolyzed xanthin and esculin, and it has susceptible for streptomycin. Owing to inhabited marine environment of this isolate, it was found has salinity tolerance up to 10%. Likewise, Sm4 isolate was found have utilized for D-glucose, D-galactose, sucrose, mannitol, raffinose, *meso*-inositol, D-fructose, and rhamnose as carbon sources, as well as L-cysteine, L-valine, L-histidin, L-alanine, L-lysine, L-leucine, L-tyrosine, L-phenylalanine, and L-proline as nitrogen sources. However, L-arabinose and xylose could not be utilized as carbon sources. All collected data that mentioned above predisposed actinomycete isolate to be similar to *Streptomyces tendae*. The confirmatory molecular identification has been carried out by using PCR technique, in which 16S rRNA gene sequence was exactly determined, which has 98% similarity to *Streptomyces tendae* M23 with accession number HM594286.1 in GenBank.

Purification of β-lactamase inhibitory protein

There were two liters of starch casein broth inoculated by *Streptomyces tendae*, and then incubated at 28°C for 7 days at 160 rpm. The inhibitory protein was precipitated by gradually supplementation of saturated ammonium sulfate (10 to 90%) to the cell free extract containing target protein. The target protein has been detected in only three active fractions at 60, 70, and 80% of ammonium sulfate concentration; however, the highest activity of target protein was observed at 70%. Subsequently, all of total activity of β-lactamase inhibitory protein (u), total protein content (mg), specific activity (u/mg), fold purification and yield per cent were calculated with each fraction to determine the highest purification which correlated with the highest value of specific activity (Table 5). Similar

results were obtained by Dale and Smith [53], Huang et al. [54], Oroszet al. [55], and Spencer et al. [56]. The active fractions were pooled and subjected to purification by using ion exchange column chromatography (diethylaminoethyl cellulose G-25 "DEAE-cellulose"), dialysis, and gel filtration column chromatography (sephadex G-200).The purified β-lactamase inhibitory protein was separated by electrophoresis atsingle clear band, which has40 KDa (Figure 3). Similar results were recorded by Black-Shear [40], and Huanget al. [54].

Antibacterial activity of target protein

The target purified protein which has inhibition effect against β-lactamase enzyme, was found has antibacterial activity toward MRSA strains. Consequently, the target protein has two or double inhibitory effect against β-lactam resistant bacteria particularly MRSA strains. Although clavulanic acid was the first clinically useful β-lactamase inhibitor, it has a broad antibacterial spectrum, encompassing both Gram-negative and Gram-positive bacteria and anaerobes [57-63].

Amino acid analysis

The amino acid sequence and content of target protein were determined using HPLC (Figure 4). The results proved that, the target protein was characterized by a high content in therionine(90 mole percent), arginine (75 mole percent) and alanine (70 mole percent). Similar results were recorded by Artette et al. [64].

Table 1: Locations of MRSA sampling stations

MRSA	Patient gender	Patient age	Infection site	Chronic diseases	Hospital
SA-1	Male	~ 45	Foot	Diabetes	Sayed Galal
SA-2	Male	~ 22	Blood	No	
SA-3	Female	~ 30	Vagina	No	
SA-4	Male	~ 40	Pubic	Diabetes	
SA-5	Male	~ 35	Pubic	No	
SA-6	Male	~ 55	Blood	No	
SA-7	Male	~ 20	Buccal cavity	No	Om El-Masreen
SA-8	Female	~ 40	Sputum	Diabetes	
SA-9	Female	~ 25	Blood	No	
SA-10	Male	~ 35	Blood	No	
SA-11	Male	~ 35	Blood	No	
SA-12	Male	~ 45	Blood	Diabetes	Dar Alfouad
SA-13	Female	~ 45	Foot	Diabetes	
SA-14	Male	~ 35	Foot	Diabetes	
SA-15	Male	~ 25	Foot	Diabetes	
SA-16	Female	~ 45	Vagina	Hypertension	Holwan Alaam
SA-17	Female	~ 20	Blood	No	
SA-18	Female	~ 40	Blood	Diabetes	
SA-19	Male	~ 40	Blood	Diabetes	Nile
SA-20	Male	~ 40	Foot	Diabetes	

Table 2: Locations of marine soils sampling stations

S.N.	Date	Depth (meters)	Location
1	6/11/2015	5	Al-Manzalah Lake
2	7/11/2015	5	Borollos Lake
3	13/11/2015	5	Abu-Qir
4	14/11/2015	5	Sidi Bishr
5	20/11/2015	5	Dahab
6	21/11/2015	5	Taba
7	27/11/2015	5	Suez
8	28/11/2015	5	Ismailia
9	4/12/2015	5	Port Said
10	5/12/2015	5	Port Fouad

Table 3: Cultural characteristics of *S.tendae*

Media	Growth rate	Color		
		Aerial mycelia	Substrate mycelia	Diffusible pigment
Tryptone yeast extract broth	Poor	264 L. Gray	79 l.gy.YBr	No
Yeast-Malt extract agar	Moderate	10 Pk. Gray	70 l.oy	No
Oat-meal extract agar	Good	263 L. Gray	76 l.y.Br	79 l.gy.YBr
Inorganic salts starch agar	Moderate	10 Pk. Gray	79 l.gy.YBr	No
Glycerol asparagine agar	Good	264 L. Gray	79 l.gy.YBr	No
Peptone yeast extract iron agar	Poor	10 Pk. Gray	79 l.gy.YBr	No
Tyrosine agar	Poor	10 Pk. Gray	79 l.gy.YBr	No

L. Gray, light gray; l.gy.YBr, light gray yellowish brown; Pk. Gray, pinkish gray; l.oy, light orange yellow; l.y.Br, light yellowish brown.

Table 4: Morphological and physiological characteristics of *S.tendae*

Parameter	Characteristic	Result
Morphology	Spore chain	Spiral
	Spore shape	Ellipsoidal
	Spore surface	Warty
	Motility	Non-motile
Biochemistry	Diaminopimelic acid (DAP)	LL-DAP
	Sugar pattern	Not detected
Physiology	Amylase, protease, pectinase, catalase and cellulase	Produced
	Lipase and lecithinase	Not produced
	Melanoid pigment production	Produced
	Degradation of xanthin and esculin	Degraded
	H_2S production	Not produced
	Nitrate reduction	Produced
	Streptomycin resistance	Sensitive
Utilization of carbon sources	D-glucose, D-galactose,Sucrose, Mannitol, Raffinose, *meso*-inositol, D-fructose, Rhamnose.	Utilized
	L-arabinose and Xylose	Not utilized
Utilization of nitrogen sources	L-cystiene, L-valine, L-histidin, L-alanine, L-lysine, L-leucine, L-tyrosine, L-phenylalanine, and L-proline	Utilized
NaCl tolerance	1 – 10%	Tolerant

Table 5: Purification table of β-lactamase inhibitory protein produced by *S.tendae*

(%) of $(NH_4)_2SO_4$	Activity (mm)	Total activity of BLIP (u)	Total protein content (mg)	Specific activity (u/mg)	Purification fold	Yield %
Filtrate	35.0	180	160	1.125	1.0	100
10%	0.00	0.00	3.5	0.00	0.00	0.00
20%	0.00	0.00	4.2	0.00	0.00	0.00
30%	0.00	0.00	4.5	0.00	0.00	0.00
40%	0.00	0.00	5.1	0.00	0.00	0.00
50%	0.00	0.00	5.5	0.00	0.00	0.00
60%	30.0	163	6.4	25.5	22.6	90.5
70%	22.0	150	6.8	22.0	19.5	83.3
80%	17.0	142	7.2	19.7	17.5	78.8
90%	0.00	0.00	7.8	0.00	0.00	0.00

BLIP = Beta-lactamase inhibitory protein

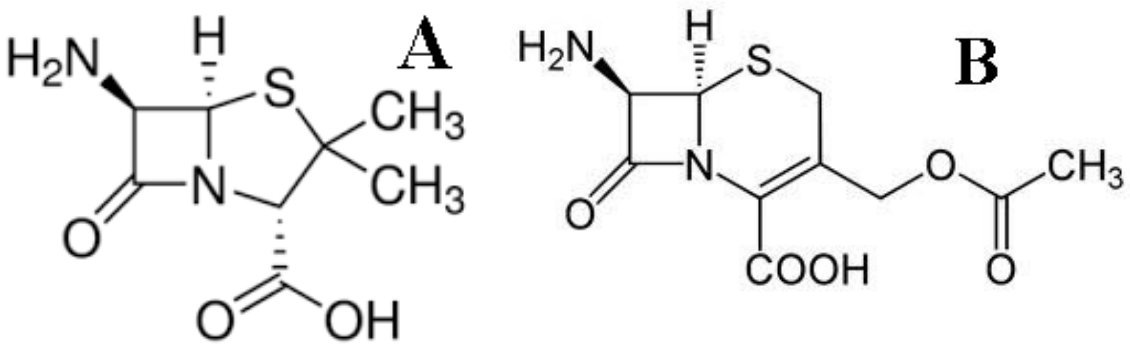

Figure 1:(A) Mother nucleus of penicillins (6-aminopenicillanic acid) and (B) Mother nucleus of cephalosporins (7-aminocephalosporanic acid)

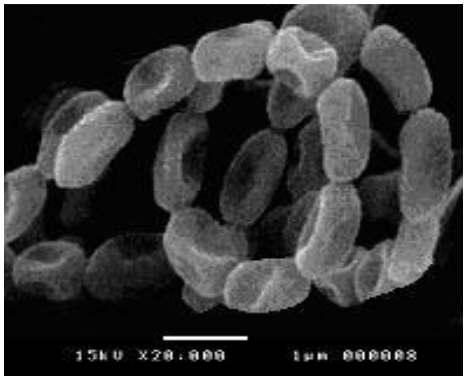

Figure 2: Scanning electron micrograph of *S. tendae*

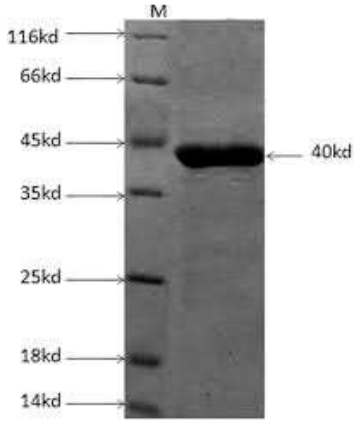

Figure 3: SDS-PAGE of β-lactamase inhibitory protein

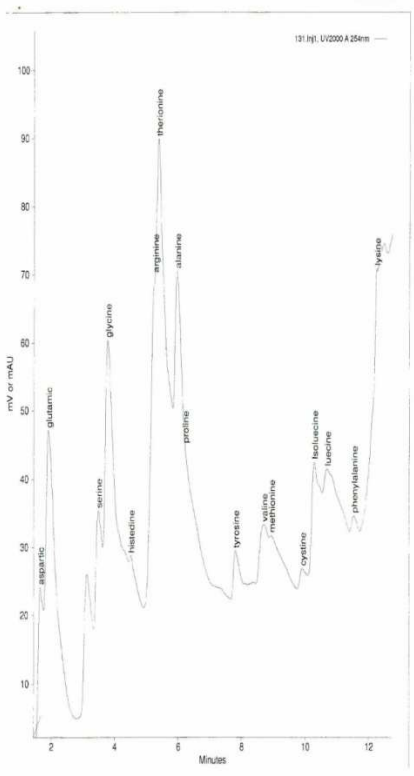

Figure 4: Amino acid analysis of β-lactamase inhibitory protein produced by *S. tendae*

CONCLUSION

Marine *Streptomyces tendae* was isolated from marine soil in Egypt. This bacterium was found has double inhibitory effect against different nosocomial strains of MRSA. Whereas, it was found produced a specific protein, which has antibacterial activity and β-lactamase inhibition activity at the same time. This protein was purified and separated at 40 KDa, and then analyzed to detect the sequence and content of amino acids. This protein was found has 17 amino acids; however, therionine, arginine and alanine were found have highest content respectively.

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