JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov 2015. Vol. **9**(Spl. Edn. 2), p. 77-86

Screening of Different Soil Sources of New York City for Antimicrobial Activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Meshari Binomar[1], Khalid I. Al Qumaizi[2], Waleed Mohammed Al Shaqha[3], Faez Iqbal Khan[4], Kathleen Bobbitt[1] and Razique Anwer[5]*

[1]Department of Biological Sciences, Wagner College: 1 Campus Road, Staten Island, New York - 10301, United States of America.
[2]Department of Family Medicine, College of Medicine, Al-Imam Mohammad Ibn Saud Islamic University, P.O.Box: 7544, Riyadh 13317-4233, Saudi Arabia.
[3]Department of Pharmacology, College of Medicine, Al-Imam Mohammad Ibn Saud Islamic University, P.O.Box: 7544, Riyadh 13317-4233, Saudi Arabia.
[4]Department of Chemistry, Steve Biko Campus, Durban University of Technology, South Africa-4001.
[5]Department of Anatomy (Microbiology), College of Medicine, Al-Imam Mohammad Ibn Saud Islamic University, P.O.Box: 7544, Riyadh 13317-4233, Saudi Arabia.

(Received: 08 August 2015; accepted: 09 October 2015)

Methicillin-Resistant *Staphylococcus aureus* (MRSA) refers to a class of bacteria that has developed resistance to the conventional antibiotics making it extremely difficult to combat. In the last few decades it has become a leading cause of nosocomial infections. Scientists and researchers agree that MRSA possesses a worldwide threat to public health and are pushed to focus more on finding a solution. The present study focuses on the *in vitro* antimicrobial activities of various microbes against MRSA commonly present in soil. Bacterial and fungal isolates obtained from different soils in New York City were evaluated for their inhibitory activities on Community Acquired MRSA. The two main plate types used for isolating microorganisms were Glycerol Yeast Extract Agar: for isolation of bacterial genera *Streptomyces* and *Bacillus*, and Saboraud Dextrose Agar: for isolation of fungal genera *Fusarium*, *Aspergillus*, and *Penicillium*. Community Acquired MRSA was plated on Trypticase Soy Agar and each of the suspected antibiotic producing microorganism was tested for inhibition of MRSA growth. However, our study reports that none of the tested microorganisms could inhibit the growth of Community Acquired Methicillin-resistant *Staphlyococcus aureus*. Suggestions and recommendations for future experiments are discussed.

Key words: Bacterial and fungal isolates**;** Methicillin-resistant *Staphlyococcus aureus***;** New York city**;** Trypticase Soy Agar.

The era of antibiotics began with the accidental discovery of "penicillin", a substance produced by the mould *Penicillium notatum*, by Alexander Fleming in 1928 that inhibited the growth of *Staphylococcus aureus*. In 1938, Howard Florey and Ernst Chain successfully followed up Fleming's work on mice models, which allowed penicillin to be used as a medicine. As a result, penicillin became one of the most effective treatments for *S. aureus*[1, 2]. In 1880, Staphylococcus was first identified in Scotland by the surgeon Alexander Ogston in pus from a surgical abscess in a knee joint. In 1884 Anton J. Roesenbach isolated *S. aureus*, known as "golden staph". *S. aureus* is a

* To whom all correspondence should be addressed.
E-mail: razainuddin@imamu.edu.sa

gram-positive coccus usually non-pathogenic and found on human skin, in the respiratory tract, and other locations in the body. However, *S. aureus* can be pathogenic depending on the bacterial strain, the host's immunity and other effects causing staphylococcal infections. With the evolution of bacterium, *S. aureus* became more hazardous in the clinical field due to their emerging resistance to antibiotics[3-6]. This requires a thorough investigation on different antibiotic resistant strains like Methicillin-resistant *S. aureus (*MRSA), which pose a worldwide threat to public health and welfare. MRSA was first recognized in the late 1960s at Boston City Hospital in the United States. MRSA is a strain of *S. aureus* that developed resistance to beta-lactam antibiotics including penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and cephalosporins. The evolution of the bacterium through the process of natural selection has resulted in development of resistance to the beta-lactam antibiotics, which work by inhibiting bacterial cell wall biosynthesis, thus limiting the treatments for MRSA[7]. MRSA produces the enzyme beta-lactamase that hydrolyses the beta-lactam ring of the antibiotic making it ineffective[8]. There are two types of MRSA: hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA). According to Centre for Disease Control and Prevention (CDC) defines HA-MRSA in persons who have had frequent or recent contact with hospitals or healthcare facilities within the previous year. If not, it is known as CA-MRSA. Although HA-MRSA and CA-MRSA are transmitted in the same fashion, both have distinct clinical differences. The number of hospital admissions for both types of MRSA has continuously increased from 1995 to 2005. In 2005 in the United States alone, there were 368,600 hospital admissions for MRSA and18,650 deaths. Till date MRSA continues to grow at an alarming rate, suggesting MRSA to be a national priority for disease control (Evans 2008). Since the ability to cure patients with MRSA is limited, scientists and researchers are pushed to focus more on finding a solution. One of the major reservoirs of microorganisms that produce antibiotics is soil. This is why in the past they relied on combinations of soil and chemicals to produce antibiotics[9-11]. For example, Streptomycin is antibiotic extracted from *Streptomyces griseus*, found usually in the soil, showed a positive treatment against tuberculosis. In addition, another antibacterial called chloromycetin was isolated from *Streptomyces venezuelae*, which has a powerful action on a wide range of infectious bacteria. Fungi are also associated with producing antibiotics such as Penicillin isolated from *Penicillium notatum*[12, 13]. Also, Cyclosporine was extracted from the fungus *Tolypocladium inflatum* that was effective against fighting bacteria[14, 15]. Therefore, using soil microorganisms to produce antibiotic has been known for long, and with MRSA complexity these soil microorganisms may be a treatment option. Exchanging genes between human pathogens and environmental bacteria by conjugation, transformation, and transduction increase the antibiotic resistance of microorganisms[16]. According to a new research study at Washington University School of Medicine "The researchers found identical genes for antibiotic resistance in soil bacteria and in pathogens from clinics around the world"[17]. Finding identical genes for antibiotic resistance in soil may lead to the evolution of other types of bacteria, which might come up with a solution to stop MRSA temporary. There are many variables that may cause changes in microbial communities in soils besides evolution such as season, climate, soil texture and other environment parameters. All of these changes in soil are reasonable enough to modify microorganisms, which then might lead to possibilities of coming up with an antibiotic for MRSA[18-20]. In the present study, we performed a thorough screening of antibiotic producing bacteria and fungi from different soil samples for Methicillin-Resistant *S. aureus* (MRSA).

MATERIALS AND METHODS

Soil Selection

Soil samples were collected from ten different places (Table 1). The collected samples were transferred to sterile plastic containers and covered with parafilm to prevent contamination. The samples were immediately taken to the laboratory.

Determination of Soil Temperature and pH

The temperatures of the soils at the ten different sites were determined using a

thermometer. The thermometer was calibrated with ice to prove its ability to work. The thermometer was inserted into the soil at up to 5 cm of depth and allowed to stay for 10 minutes, after which the temperature reading was obtained. The average of three consecutive readings was recorded for each site. Hydrion Papers were used to measure the soils' pH. 1 g of the soil in natural solution (distilled water) were taken pH measurement and stirred for 5 seconds. Then, the Hydrion Paper was inserted into the solution. The pH of the soil was tested three times, and the average of the three readings was recorded. Citric acid (lemon) solution was used as a control to prove the Hydrion Papers ability to work properly.

Isolation of Bacteria and Fungi from soil samples

Soil samples were incubated for 3-4 hour at 45°C, crushed and sieved prior to use. Different types of culture media were used to isolate an antibiotic producing bacteria or fungi from the soils. Glycerol Yeast Extract Agar (GYEA) was selected for the isolation of *Streptomyces* and *Bacillus* because they are very dominant in the soil and capable of producing an antibiotic[21]. Saboraud Dextrose Agar (SDA) was selected for the isolation of *Dermatophytes* which are known for antibiotic producing treatment[22].

Identification of Bacteria

Gram Stain was done for identifying *Streptomyces* and *Bacillus*. *Streptomyces* is a Gram-positive filamentous rod shaped bacteria. *Bacillus* is a Gram-positive rod shaped, and it produces oval endospores. Other microorganisms were eliminated from the experiment[23]. Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen. Catalase should be positive or weakly positive for proving an identification of *Streptomyces* and *Bacillus*, so any negative results were eliminated from the experiment[24]. Based on previous research, *Streptomyces* must be positive in Starch Hydrolysis, Casein Hydrolysis, Citrate, H_2S Production, Sucrose Test, and Oxidase Test for proving its category. For *Bacillus* identification: Litmus Milk, Trypticase Soy Agar (TSA), Blood Agar, Starch Hydrolysis, Nitrate Test, Citrate Test, Motility Test, Indole Test, Glucose Test, Lactose Test, and Oxidase Test were done[25].

Identification of Fungi

Lactophenol Cotton Blue (LPCB) wet mounts were used for staining and observing fungi. A clean glass slide on a sheet of white paper was kept and a small drop of Lactophenol Cotton Blue was added to the slide. The adhesive side of a small length of transparent tape was touched to the surface of the colony. The length of the tape adhered to the surface of the slide with the Lactophenol Cotton Blue. Finally, the slide were examined microscopically [26].

Identification of CA-MRSA

CA-MRSA was taken from Wagner College Laboratory. TSA was used for growing CA-MRSA strains for three days. *S. aureus* was identified using a microscope, chemical tests, Mannitol salt agar and by using Penicillin and Vancomycin to prove if the bacterium was Methicillin-resistant. In case of Penicillin with MRSA, no zone of inhibition should be obtained. However, Vancomycin should provide a zone of inhibition with MRSA[27].

Table 1. Soil sites, temperature, and pH

Soil site	Location	Temperature	pH
A	Union Building, Wagner College	24 °C	5.8
B	Spiro Sports Center, Wagner College	21 °C	5.2
C	Harborview Residence Hall, Wagner College	24 °C	5
D	Megerle Science Hall, Wagner College	21 °C	5.6
E	Sutter Oval, Wagner College	18 °C	5.8
F	Strawberry fields, Central Park	29 °C	5.2
G	Cedar Hill, Central Park	30 °C	5.2
H	Major Ave., Staten Island	22°C	6
I	Clove Lake Park	20°C	5.2
J	Clove Lake Park	21°C	5

Screening of Streptomyces and Bacillus strains against MRSA strain

The screening method of isolates involved two steps: primary and secondary screening. In primary screening, antimicrobial activity of pure isolates was identified by cross streak method. Plates were incubated at 28°C for 7 days. In secondary screening method, screening of isolates was done by disc diffusion method. A sterile cotton swab was dipped into the suspension and excess fluids were removed. The inoculums were spread evenly over the entire surface of the plate by swabbing in three directions. The plates were inverted and incubated[28]. Cell concentration of test organisms were adjusted at 0.5 McFarland turbidity standards, which has been used against MRSA strain for antimicrobial susceptibility.

RESULTS

Soil Selection

All soil samples were collected from New York City (Table 1). The locations of the soil collection were: five from Wagner College, two from Central Park, two from Clove Lake Park, and one from Major Avenue Playground (Staten Island) (Table 1).

Determination of Soil Temperature and pH

After the thermometer was tested with ice in the laboratory and showed a 0°C result, the average of three consecutive readings was recorded for each soil site (Table 1). The variations in the temperature were seen in the sample due to

Table 2. Microorganisms Isolation to GYEA or SDA

Soil Site	GYEA (Bacteria)	SDA (Fungi)
A	3 Colonies	0 Colony
B	2 Colonies	3 Colonies
C	0 Colony	0 Colony
D	2 Colonies	3 Colonies
E	3 Colonies	2 Colonies
F	1 Colony	2 Colonies
G	2 Colonies	0 Colony
H	3 Colonies	1 Colony
I	0 Colony	2 Colonies
J	1 Colony	3 Colonies

Table 3. Gram Stain & Catalase results for Bacterial Identification

Bacteria	Gram Stain	Catalase	Bacteria	Gram Stain	Catalase
A1	GNC	Not Done	E3	GPR (Endospore)	+
A2	GPR	+	F1	GPR (Endospore)	W +
A3	GPR	+	G1	GPC	Not Done
B1	GPR (Endospore)	W +	G2	GPR	+
B2	GPR	-	H1	GPR(Endospore)	+
D1	GPR (Endospore)	W +	H2	GPR	W +
D2	GPR (Endospore)	+	H3	GPR(Endospore)	Weak +
E1	GNR	Not Done	MRSA(Control)	GPC	+
E2	GPR (Endospore)	W +			

Positive (+), Negative (-), Weak Positive (W +), MRSA used as a control, GPR (Gram Positive Rods), GNR (Gram Negative Rods), GPC (Gram Positive Cocci), GNC (Gram Negative Cocci)

Table 4. *Streptomyces* Identification

Bacteria	Oxidase	Starch Hydrolysis	Casein Hydrolysis	Citrate	H_2S Production	Sucrose Test
A2	+	+	+	+	+	+
A3	+	-	-	+	-	+
B2	+	+	+	+	+	+
G2	+	+	+	+	+	+
H2	+	+	+	-	+	+

different weather conditions. The measurements were based on a vision comparing between the closest two pH numbers using Hydrion paper. After citric acid (lemon) showed a pH of 3, which was used as a control, the average of three consecutive readings was recorded for each soil sample (Table 1).

Growth and Identification of CA-MRSA

CA-MRSA were grown on Tryptocase Soy Agar (TSA) and left at room temperature (Fig. 1). *S. aureus* was identified using microscopy, biochemical tests, and Mannitol Salt Agar. By using the Gram Stain method, the isolated bacterium from TSA showed Gram-positive cocci in clusters. The Catalase and Coagulase tests showed positive results. The bacterium fermented mannitol in Mannitol Salt Agar that caused the phenol red in the agar to turn yellow (Fig. 2). In addition, using Penicillin and Vancomycin was important to prove if the bacterium was MRSA. In the case of Penicillin with the MRSA, it provided no zone of inhibition. However, Vancomycin provided a zone of inhibition with MRSA (Fig. 3).

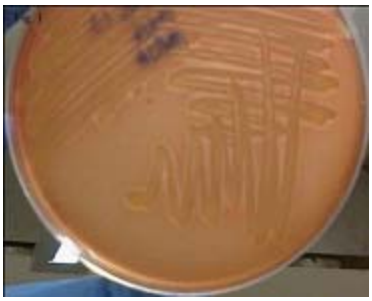

Fig. 1. *Staphylococcus aureus* in TSA

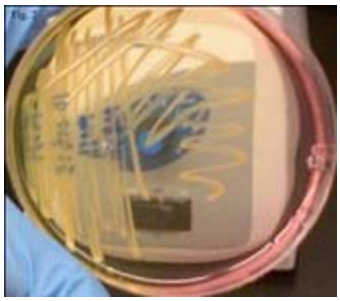

Fig. 2. *Staphylococcus aureus* in Mannitol Salt Agar

Table 5a. *Bacillus* Identification

Bacteria	Hemolysis	Litmus Milk	TSI	Starch Hydrolysis	Oxidase
B2	Alpha	Acid	A/A	+	+
D1	Beta	Alkaline	A/K	+	-
D2	Beta	Alkaline	A/A	+	+
E2	Beta	Acid	A/A	+	+
E3	Gamma	Acid	A/A	-	+
F1	Beta	Alkaline	A/K	+	+
H1	Alpha	Alkaline	A/K	+	-
H3	Beta	Alkaline	A/K	+	+

TSI (Triple Sugar Iron), A/A (Acid/Acid), A/K (Acid/Alkaline)

Table 5b. *Bacillus* Identification

Bacteria	Motility	Indole Test	Glucose Test	Lactose Test	Citrate
B2	+	-	+	+	-
D1	+	-	+	-	-
D2	+	-	+	-	-
E2	+	-	+	+	-
E3	+	-	+	+	-
F1	+	-	+	-	-
H1	+	-	+	-	-
H3	+	-	+	-	-

Isolation of Bacteria and Fungi from Soil Samples

GYEA and SDA were made after the dilutions of all soil samples were done. GYEA was selected for the isolation of *Streptomyces* and *Bacillus.* The GYEA media produced a total of 16 colonies from all soil samples (Fig. 4). SDA was selected for the isolation of Dermatophytes, and all SDA media gave 11 colonies from all soil samples (Fig. 5). The results of all microorganisms isolation of GYEA or SDA were recorded (Table 2). For identification, each microorganism was isolated individually using GYEA or SDA, depending on the colony category. For example, in soil sample (I) there were two colonies grown on the SDA (Fig. 6). Each colony was isolated to its own separate media for identification purposes, and each colony was numbered such as I1 and I2 to simplify the process (Fig. 7). The same method was done with each sample in the experiment.

Identification of Bacteria

A Gram Stain was done to identify *Streptomyces* and *Bacillus* since they are very

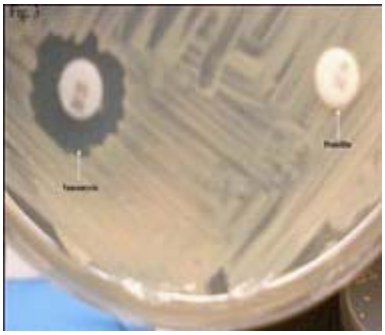

Fig. 3. Penicillin and Vancomycin on MRSA

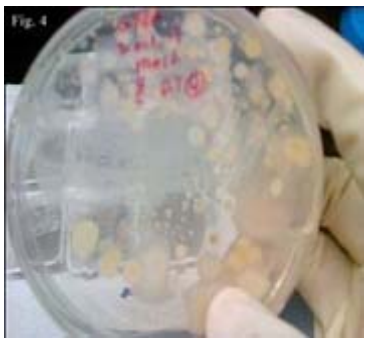

Fig. 4. GYEA with various growing bacteria

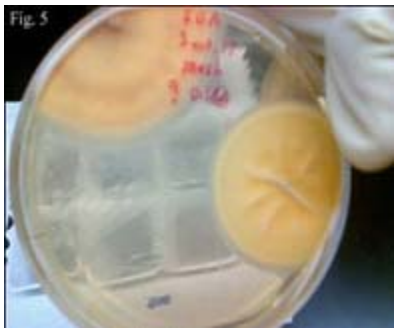

Fig. 5. SDA with two fungi growing on the media

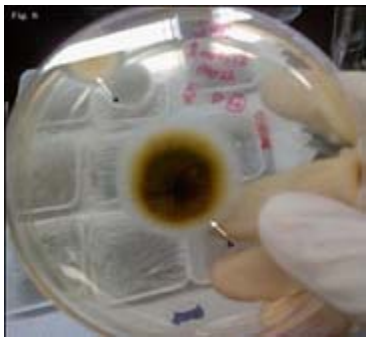

Fig. 6. Soil sample (I) had two fungi growing

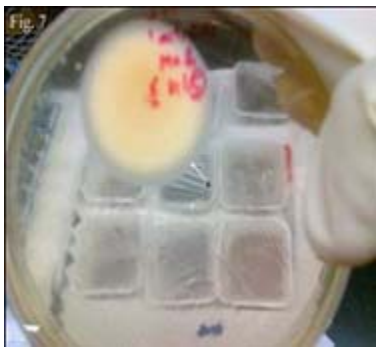

Fig. 7. Fungus (I2) isolated to new SDA plate

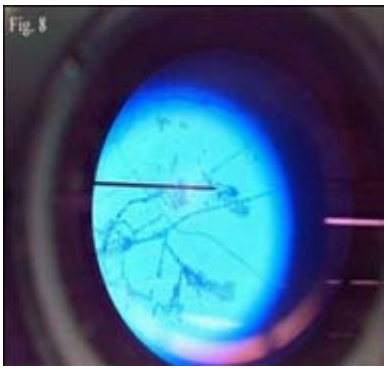

Fig. 8. Fungal strains in LPCB wet mount staining

common antibiotic producers. *Streptomyces* is a Gram-positive rod (filamentous rod shaped), and *Bacillus* is a Gram-positive rod with endospore. Also, Catalase was done to prove the identification of *Streptomyces* and *Bacillus* which both have mostly positive or weak positive results (Table 3) (15); (16). The CA-MRSA that was taken from Wagner College Laboratory that was used as a control for the Gram Stain and Catalase Test. *Streptomyces* must be positive in Starch Hydrolysis, Casein Hydrolysis, Citrate, H_2S Production, Sucrose Test, and Oxidase Test for proving its identification as Swami Ramanand Teerth Marathwada University mentioned. These tests were prepared for suspected *Streptomyces* samples, and the results show all positive except in sample A3 and H2 (Table 4). The results showed that colony A3 was not *Strepotmyces* since it gave negative results with Starch Hydrolysis, Casein Hydrolysis, and H_2S Production. Colony H2 showed all positive results except with Citrate which was questionable. The Citrate test was

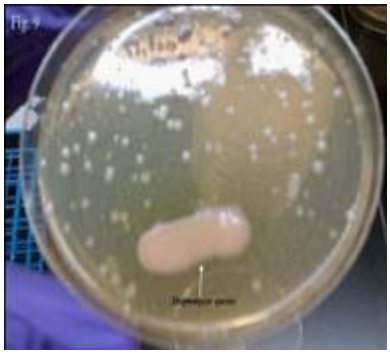

Fig. 9. *Streptomyces* in TSA planted with MRSA

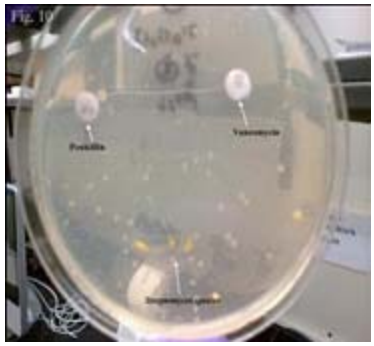

Fig. 10. *Streptomyces* in TSA planted with MRSA

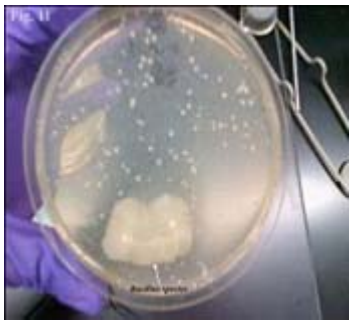

Fig. 11. *Bacillus* in TSA planted with MRSA

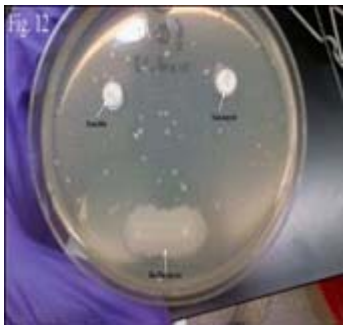

Fig.12. *Bacillus* in TSA planted with MRSA

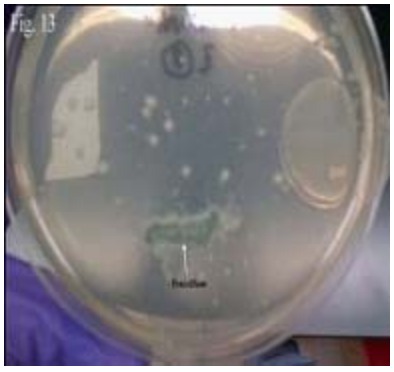

Fig. 13. *Penicillium* in TSA planted with MRSA

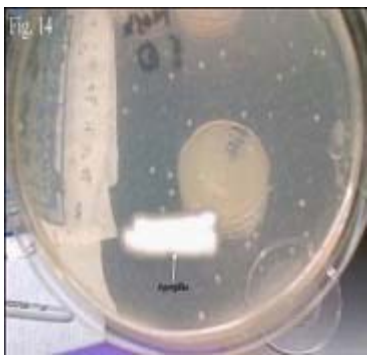

Fig. 14. *Aspergillus* in TSA planted with MRSA

repeated three times with H2 colony, and the test gave the same result each time. Therefore, H2 needs a further identification by using reverse PCR to prove its genus, which was not done in this experiment. According to the Virtual Microbiology book in chapter eleven (Isolation of *Bacillus*) recommends the use of Litmus Milk, Trypticase Soy Agar (TSA), Blood Agar, Starch Hydrolysis, Nitrate Test, Citrate Test, Motility Test, Indole Test, Glucose Test, Lactose Test, and Oxidase Test for the identification of *Bacillus*. These tests were prepared for suspected *Bacillus* samples, and the results were recorded (Table 5a and 5b). A further identification by using reverse PCR to prove its genus is recommended.

Identification of Fungi

LPCB wet mount was used for observing fungi (Fig. 8). The results showed seven *Penicillium* strains, five *Fusarium* strains, and four *Aspergillus* strains. Further identification with reverse PCR could be done to prove the fungus genus and species, which was not done in this experiment. Also, the SDA was selected to isolate Dermatophytes, but unfortunately no microorganisms were grown. Therefore, zoo, sewer, and pond soils should have been considered instead of only soils from locations such as parks.

Antibiotic Producing Bacteria and Fungi from Soils

The research was based on the ability of bacteria and fungi to produce antibiotics specific to CA-MRSA. There were 17 colonies isolated from GYEA, eight of them were *Bacillus*, and five *Streptomyces*. All of the bacterial colonies isolated showed negative antibiotic production against CA-MRSA (Fig. 9, 10, 11 and 12). In addition, there were 16 microorganisms isolated from SDA that showed negative results against CA-MRSA, five were identified as *Fusarium*, four as *Aspergillus*, and seven as *Penicillium* (Fig. 13 and14). As a result, from the selected soils there was no antibiotic producing bacteria and fungi for CA-MRSA.

DISCUSSION

More than 30 species of Staphylococci can infect humans, but Staphylococci in majority of cases are normally found on the skin of around 25% to 30% of healthy adults and less in hospital worker by approximately 25%. However, infection can occur easily when damage to the skin allows the bacteria to invade the human body and defeat its protection mechanism, or by more complex methods Staphylococci can infect human like surgical implants. *S. aureus* is considered one of the most common/frequent/widespread causes of infections in general, but certain groups of people at higher risk including newborn, infants and people with chronic conditions such as cancer and diabetes. These high risk conditions may lead *S. aureus* to be lethal in several cases[29]. *S. aureus* has become increasingly prevalent and risky since 1980s, and more than 95% of patients worldwide are not responding to first-line antibiotics as penicillin[30]. The increase of MRSA is continually over the years in 1974 only 2% of all *S. aureus* infections were MRSA, in 1995 it was 22%, and in 2004 became 64% with an estimate of increasing the number up to 70% currently. According to the Centers for Disease Control and Prevention (CDC), estimated around 94,360 patients were infected with MRSA, but other organizations estimated over one million infect with MRSA only in the US in 2005. In the same year, the healthcare system spent more than $9.7 billion on MRSA infections only not taking into account indirect costs such as time spent in the hospital (Hannah B, n.d). The average cost per CA-MRSA infection is $7,070 to $20,489, which is higher by two to five times than the cost of influenza case, and three to ten times than the cost of food borne illness or pertussis. Therefore, the high number of infections and costs should guide scientists and researchers to focus more on preventing and controlling MRSA to avoid a health disaster in future[31]. Vancomycin is antibiotic that was isolated from *Amycolatopsis orientalis* found in a soil sample. In 1958, vancomycin used for clinical treatment against penicillin-resistant *S. aureus*, but the antibiotic remained limited to patients with allergy and MRSA infection. After 1980, vancomycin became the main treatment for MRSA however in 2002 fifteen confirmed cases of vancomycin resistant *S. aureus* had been reported in three different countries including US, India, and Iran[32]. Daptomycin is another antibiotic that is isolated from soil and used to treat MRSA. Daptomycin was extracted from *Streptomyces roseosporus* found in a soil sample from Mount Ararat (Turkey). Daptomycin was chosen for

treatment because of its *in vivo* efficacy in animals, and in 2003 the antibiotic was approved by the FDA[33]. Based on study was done at the University of Lagos (Nigeria) to determine the presence and types of antibiotic-producing bacteria and fungi, they found these following species are the most common antibiotic producers: *Bacillus licheniformis*, *Bacillus subtilis*, *Penicillium chrysogenum*, *Streptomyces reticuli*, and *Streptomyces hygroscopicus*[9]. This is the reason we focus on our study to screen for *Bacillus*, *Streptomyces*, *Fusarium*, *Aspergillus*, and *Pencillium* since they are really known for antibiotic producing microorganisms. Unfortunately, our laboratory results showed all microorganisms isolated from soil gave negative results for antibiotic production against CA-MRSA. However, this does not mean there is no future treatment for CA-MRSA; in fact scientists and researchers must work together and harder to protect and prevent the society from the first health care future concern MRSA. Since the CA-MRSA was taken from Wagner College. The chance of finding an antibiotic for CA-MRSA was higher if the soil was selected from the same area[10]. This explained why five samples were selected from Wagner College. The rest of the soil collections were randomly chosen, but at the same time focused on where most people can be located. CA-MRSA was plated on TSA then all suspected antibiotic producing microorganisms (*Bacillus*, *Streptomyces*, *Fusarium*, *Aspergillus*, and *Pencillium*) were tested to inhibit CA-MRSA growth. Even though the results of our study were negative for antibiotic producing, more selected and variety of soil will provide a higher chance of finding antibiotic producing for a future research recommendation. In conclusion since the research depends on both bacterial and fungal antibiotic producers, GYEA and SDA were used. The GYEA isolated bacteria included *Bacillus* and *Streptomyces*. Also, the SDA isolated fungi included *Fusarium*, *Aspergillus*, and *Penicillium*. All microorganisms isolated from GYEA and SDA gave negative results for antibiotic production against CA-MRSA that might be because isolates were not able to reach intracellularly and non-denaturation of the bacterial cell wall of MRSA strain. Screening of antimicrobial activity of isolates against MRSA strain has been done by cross streak method for primary screening and disk diffusion method for secondary screening. Although this methodology is easier, standard and its advantages are i) low cost, ii) ease in modifying test antimicrobial disks when required, iii) can be used as a screening test against large numbers of isolates, iv) can identify a subset of isolates for further testing by other methods, such as determination of MICs as compared to other methodology such as cell lysate, crude extract.

ACKNOWLEDGMENTS

The authors wish to acknowledge Al-Imam Muhammad Ibn Saud Islamic University, Riyadh for providing financial support. We would like to express our gratitude to his colleagues Shama Parween from Institute of Genomics and Integrative Biology, India.

REFERENCES

1. Ligon, B.L., Penicillin: its discovery and early development. *Semin Pediatr Infect Dis,* 2004. **15**(1): p. 52-7.
2. Diggins, F.W., The true history of the discovery of penicillin, with refutation of the misinformation in the literature. *Br J Biomed Sci,* 1999. **56**(2): p. 83-93.
3. Kotilainen, P., et al., Elimination of epidemic methicillin-resistant Staphylococcus aureus from a university hospital and district institutions, Finland. *Emerg Infect Dis,* 2003. **9**(2): p. 169-75.
4. Madani, T.A., Epidemiology and clinical features of methicillin-resistant Staphylococcus aureus in the University Hospital, Jeddah, Saudi Arabia. *Can J Infect Dis,* 2002. **13**(4): p. 245-50.
5. Layton, M.C., W.J. Hierholzer, Jr., and J.E. Patterson, The evolving epidemiology of methicillin-resistant Staphylococcus aureus at a university hospital. *Infect Control Hosp Epidemiol,* 1995. **16**(1): p. 12-7.
6. Missiakas, D.M. and O. Schneewind, Growth and laboratory maintenance of Staphylococcus aureus. *Curr Protoc Microbiol,* 2013. Chapter **9**: p. Unit 9C 1.
7. Gurusamy, K.S., et al., Antibiotic prophylaxis for the prevention of methicillin-resistant Staphylococcus aureus (MRSA) related complications in surgical patients. *Cochrane Database Syst Rev,* 2013. **8**: p. CD010268.
8. Drawz, S.M. and R.A. Bonomo, Three decades

of beta-lactamase inhibitors. *Clin Microbiol Rev*, 2010. **23**(1): p. 160-201.
9. Adeleye, I.A., S. Eruba, and C.J. Ezeani, Isolation and characterisation of antibiotic producing microorganisms in composted Nigerian soil. *J Environ Biol*, 2004. **25**(3): p. 313-6.
10. Srividya, A.R., G.S. Saritha, and B. Suresh, Study of the soil isolates for antimicrobial activity. *Indian J Pharm Sci*, 2008. **70**(6): p. 812-5.
11. Khan, F.I., et al., Thermostable chitinase II from Thermomyces lanuginosus SSBP: Cloning, structure prediction and molecular dynamics simulations. *J Theor Biol*, 2015. **374**: p. 107-14.
12. Frey-Klett, P., et al., Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiol Mol Biol Rev*, 2011. **75**(4): p. 583-609.
13. Stephens, D.E., et al., Creation of thermostable and alkaline stable xylanase variants by DNA shuffling. *J Biotechnol*, 2014. **187**: p. 139-46.
14. Bushley, K.E., et al., The genome of tolypocladium inflatum: evolution, organization, and expression of the cyclosporin biosynthetic gene cluster. *PLoS Genet*, 2013. **9**(6): p. e1003496.
15. El Enshasy, H., et al., Kinetics of cell growth and cyclosporin A production by Tolypocladium inflatum when scaling up from shake flask to bioreactor. *J Microbiol Biotechnol*, 2008. **18**(1): p. 128-34.
16. Dantas, G. and M.O. Sommer, Context matters - the complex interplay between resistome genotypes and resistance phenotypes. *Curr Opin Microbiol*, 2012. **15**(5): p. 577-82.
17. Davies, J. and D. Davies, Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev*, 2010. **74**(3): p. 417-33.
18. Pasternak, Z., et al., Spatial and temporal biogeography of soil microbial communities in arid and semiarid regions. *PLoS One*, 2013. **8**(7): p. e69705.
19. Shaffer, R.K., The challenge of antibiotic-resistant Staphylococcus: lessons from hospital nurseries in the mid-20th century. *Yale J Biol Med*, 2013. **86**(2): p. 261-70.
20. Anwer, K., et al., Role of N-terminal residues on folding and stability of C-phycoerythrin: simulation and urea-induced denaturation studies. *J Biomol Struct Dyn*, 2015. **33**(1): p. 121-33.
21. Chaudhary, H.S., et al., Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). *J Adv Pharm Technol Res*, 2013. **4**(2): p. 118-23.
22. Youssef, N., C.H. Wyborn, and G. Holt, Antibiotic production by dermatophyte fungi. *J Gen Microbiol*, 1978. **105**(1): p. 105-11.
23. Lee, H.A. and J.H. Kim, Isolation of Bacillus amyloliquefaciens Strains with Antifungal Activities from Meju. *Prev Nutr Food Sci*, 2012. **17**(1): p. 64-70.
24. Bhasin, S. and H.A. Modi, Optimization of Fermentation Medium for the Production of Glucose Isomerase Using Streptomyces sp. SB-P1. *Biotechnol Res Int*, 2012. **2012**: p. 874152.
25. Logan, N.A. and R.C. Berkeley, Identification of Bacillus strains using the API system. *J Gen Microbiol*, 1984. **130**(7): p. 1871-82.
26. Leck, A., Preparation of lactophenol cotton blue slide mounts. Community Eye Health, 1999. **12**(30): p. 24.
27. Hecht, G.B., P. Mosto, and C.S. Slater, Effectiveness of an applied microbiology course specifically designed for chemical engineering majors. *Microbiol Educ*, 2003. **4**: p. 13-22.
28. Darabpour, E., et al., Isolation of a potent antibiotic producer bacterium, especially against MRSA, from northern region of the Persian Gulf. *Bosnian Journal of Basic Medical Sciences*, 2012. **12**: p. 108-121.
29. Lowy, F.D., Staphylococcus aureus infections. *N Engl J Med*, 1998. **339**(8): p. 520-32.
30. Rubin, R.J., et al., The economic impact of Staphylococcus aureus infection in New York City hospitals. *Emerg Infect Dis*, 1999. **5**(1): p. 9-17.
31. Lee, B.Y., et al., The economic burden of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA). *Clin Microbiol Infect*, 2013. **19**(6): p. 528-36.
32. Dhand, A. and G. Sakoulas, Reduced vancomycin susceptibility among clinical Staphylococcus aureus isolates ('the MIC Creep'): implications for therapy. F1000 *Med Rep*, 2012. **4**: p. 4.
33. Eisenstein, B.I., F.B. Oleson, Jr., and R.H. Baltz, Daptomycin: from the mountain to the clinic, with essential help from Francis Tally, MD. *Clin Infect Dis*, 2010. 50 Suppl 1: p. S10-5.