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RESEARCH ARTICLE OPEN ACCESS

Characterization of Methicillin-resistant *Staphylococcus aureus* Isolated from Nearby Hospitals from two Different Countries

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is non-motile, facultatively anaerobic, gram-positive coccus non-sporulating bacterium. The MRSA was isolated as coagulase and catalase-positive, forming yellow-golden colonies on nutritive media as well as rosy to mauve colonies on CHROM-agar. MSRA is found in the mucus of the human, human skin, nasal cavity as an opportunistic pathogen and cause death in a person with a weak immune system and show resistant to the antibiotic methicillin. The MRSA is distributed greatly in clinics and hospitals, where it grows on surfaces of walls and benches. *Staphylococcus* bacteria are not restricted to animals and/or human beings but also found in the environment such as plant surfaces, and wastewater. *S. aureus* which shows resistance against methicillin is known to survive in the hospital environment for long periods and are found on the floor and walls of the building. However, before the present study, no reports have been found about the availability of MSRA in the soil. Finding of the present study would be critical as it would give a definite source that how MRSA was carried from the human to the environment of hospitals.

Keywords: Methicillin-resistant, *Staphylococcus aureus*, Pathogenic bacteria, Antibiotic resistance.

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium belongs to the Gram-positive group of bacteria and is famous for its significant role as multi-drug resistant[1] .It was isolated from the skin of human being and identified in 1961 from a hospital in the United Kingdom. MRSA causes several, which are difficult to treat because the MRSA is resistant to multiple drugs, which makes it the most hazardous strain. MRSA has developed, multidrug resistance against ג-lactam antibiotics through natural selection and horizontal gene transfer.

Staphylococci offered resistance against methicillin because of having staphylococcal cassette chromosomes (SCC*mec*), consisting *mec* A gene which codes for penicillin-binding protein and hence the strain become resistant to methicillin. Antibiotic-containing ג-lactam is common and frequently used a broad range of pathogens. The pathogens which offer resistance against these group antibiotics are called methicillin-resistance *S. aureus* (MRSA), while those which are susceptible are called methicillin-susceptible S. aureus (MSSA). The resistance of the bacterial strain depends on environmental factors and contaminants to which it is exposed, that is the reason that more resistant strains are found in the polluted area[2].

Antibiotic resistance developed in microbe has been a major problem all over the world. The records of early 1940 showed that about 60% of bacterial strains had been resistant against penicillin, acquired from hospitals[3-6]. However, the resistance has been now dropped down to 10% in hospital-acquired bacterial against synthetic penicillin[7]. Beta-lactamase hydrolysis e.g., methicillin were developed in the 1960s to cope with bacterial resistances against conventional penicillin but bacteria got resistance against these antibiotics too very soon. A good example of methicillin bacteria has been *Staphylococcus aureus* known as methicillin-resistant *S. aureus* (MRSA). A Large number of isolated pathogens from hospitals form worldwide showed methicillin resistance due to a novel penicillin-binding protein[8]. Vancomycin has been the only effective drug against the Staphylococcal infections, which has been considered as last line of defense against *S. aureus*. In Japan, *S. aureus* isolated from different hospitals revealed a gradual decline in susceptibility against vancomycin has been reported. The resistances of MSRA isolated from Japanese hospitals against vancomycin were reached to 20%[9]; and the resistance has been thought to increase in many countries.

Molecular typing technique is being used to establish relationships among the bacterial strains along with identification of internationally spread of MRSA strains.

Previous studies reported that multi-drug resistance of in MRSA was gradually increased different area and different countries[10-11]. The multi-drug resistance of the MRSA greatly depends on the location (ecotype) and types of pollutants[12]. MRSA strains isolated from hospital sewage and fecal sewage were identified as most resistant against a wide range of antimicrobial drugs. It showed that the agents conferring antibiotic resistance were not present only in pathogenic strains but also in strains isolated from environmental sewages [13].

Environmental conditions of the hospitals are presumably less susceptible to some frequently used disinfectant and strains got resistance against this disinfectant and antibiotics. The exposure of antibiotic and disinfectants may result in the development of more antibiotic resistant genes in the bacteria and became resistant against those antibiotics, which have been effective in the past. The pieces of evidence regarding reduced susceptibility antibiotic came from the exposure of these, however, it is assumed that assume that in an environment of clinic and hospitals, microbes may be exposed to some amount of disinfectant remaining on surfaces and/or even on skin or mucosae. The present study aimed to determine MRSA present in soils of nearby hospitals and determine the correlation of existence of MRSA in soil samples from two different countries.

MATERIALS AND METHODS

Collection of sample

Exactly 100 g samples (soil) were collected during spring 2018 from the nearby area of Sheffield hospital UK and vicinity of hospitals Jeddah, Saudi Arabia. The samples were collected from a radius of 750 meters. After collection, samples were packed in sterilized polyene bags and shifted to labs.

Isolation of Bacterial

Analysis of biological activities was performed using serial dilution by mixing 1 g sample in to 99 ml sterile distilled water. The mixture was shaken well and at 70 rpm for 15 min and then subjected to serial dilution at the rate of 10^{-3} to 10^{-7} and 0.1 ml was spread over the HROM-agar plates. The culture plates were incubated in triplicate at 30°C for 18-24 h. The MRSA colonies appeared as rose-mauve in mixed culture. Where many of them were colorless, blue and or other colors of bacterial colonies. Moreover, the colonies were confirmed by picking and transferred to new plate of CHROM-agar. Further, these colonies were then transferred to produce a lawn on Mueller Hinton agar.

Antibiotic assessment

The antibiotic sensitivity of the isolated bacteria was tested by disc fusion method. The discs (Oxoid) were put in the middle of the plate. The plates were then incubated at 37°C for 48 h. Any presumptive MRSA was checked to determine if they were acid fast and coagulase positive, using standard methods [14-15]. The Antibiotics used in this study was Aztreonam (20 mg/L), ciprofloxacin (8 mg/L) and colistin sulphate (1000000 U/L).

RESULTS AND DISCUSSION

Mauve colonies on CHROM-agar were isolated as putative MRSA and were further confirmed to be cocci in grape-like structure (*Staphylococcus*) through light microscopic examination. The biochemical analyses revealed that the strains were Gram-positive, coagulase positive and non-acid fast, indicated the isolates were *S. aureus.* Further, the strains were confirmed to be MRSA as they were resistant against methicillin and penicillin.

MRSA was isolated from all of the soils around the different hospitals of Jeddah - Saudi Arabia and the UK (in a radius of 750 meters) as listed in tables (Table 1 and 2). Particularly Table 1 consisted MRSA isolated for hospitals in Jeddah. The data revealed that there was a great variation in the bacteria isolated from different soil samples, which were dependent on distances among the hospitals. Greater the distance, more variation among the bacterial type. However, an abundance of MRSA in the soil of each hospital was consistent and a decrease was found in the number of MRSA except East Jeddah General hospital where the MSRA was comparatively reduced in number. While soil samples from Al Hijaz General Medical Clinic and the Jeddah National hospital (North) contains a large numbers of MRSA.

The abundance of MRSA as seen in (Table 1) may be found in densely populated areas with poor medical facilities, and improper sewage and poor hygienic practices. The large numbers of MRSA might be due to the highly dense population per unit area with poor access to hygienic and medical facilities [16]. The other reason for the large number of MRSA could be the greater numbers of medical centers with heavy rush of patients and career.

Results (Table 2) revealed the collection list of MRSA, isolated from surrounding areas of two hospitals located in Sheffield UK. The Bacterial population of antibiotic resistance was found in Saudi Arabian soil. The existence antibiotic resistance in Saudi soil was significantly greater as compared to the soils of UK. The reason could be

Table 1. Existence of MRSA in soils nearby the hospitals in Jeddah

Jeddah Hospitals	10 m	250 m	750 m
Al Hijaz General Medical Clinic (Centre)	$16.1 \times 10^5 \pm 5.4 \times 10^2$	$12.1 \times 10^5 \pm 2.3 \times 10^2$	$10.1 \times 10^5 \pm 5.1 \times 10^2$
Aljehani Hospital (South)	$1.2 \times 10^5 \pm 0.1 \times 10^2$	$0.8 \times 10^5 \pm 3.5 \times 10^2$	$0.5 \times 10^5 \pm 0.8 \times 10^2$
East Jeddah General Hospital (East)	$12.1 \times 10^5 \pm 3.1 \times 10^2$	$0.5 \times 10^5 \pm 0.5 \times 10^2$	$0.9 \times 10^5 \pm 0.5 \times 10^2$
Dr.Soliman Fakeeh Hospital (West)	$1.2 \times 10^5 \pm 0.6 \times 10^2$	$1.1 \times 10^5 \pm 0.3 \times 10^2$	$0.7 \times 10^5 \pm 0.5 \times 10^2$
Jeddah National Hospital (North)	$13.9 \times 10^5 \pm 1.5 \times 10^2$	$1.5 \times 10^5 \pm 0.5 \times 10^2$	$2 \times 10^5 \pm 0.1 \times 10^2$

Table 2. Existence of MRSA in samples of soils collected near the hospitals in Sheffield

Sheffield Hospitals	10 m	250 m	750 m
Northern General Hospital	$10.4 \times 10^2 \pm 6.1$	$19.9 \times 10^2 \pm 9.9$	$2.2 \times 10^2 \pm 11.1$
Weston Park Hospital	$18.9 \times 10^2 \pm 2.2$	$4.9 \times 10^2 \pm 5.5$	$2.1 \times 10^2 \pm 2.9$

topographic and environmental conditions of UK, which facilitate the wash away of bacteria to lower surface through rainfall. The difference in MRSA strains in both places could be the sampling points, which were different from each other in term of soil type, horticultural treatment, and vegetation cover and most important environmental and climate [17]. It was not therefore, astonishing that number of bacteria generally and MRSA especially were varied in the term of antibiotic resistance. However, there had been no association found number of MRSA isolates and proximity of the hospitals [18].

A bacterial strain of *S aureus* was isolated from the adjacent area of the Weston Park Hospital was found to be greatly resistant against antibiotics such as ampicillin and/or penicillin G. all the tow antibiotics (ampicillin, penicillin G) were beta-lactam antibiotics which act as substitutions for methicillin. It was a matter of seriousness that those such antibiotic-resistant bacteria were present in soils of Sheffield hospitals. Member of the Staphylococci, such as *S. aureus* has been found to be very active and can be survived at extremely high rates in the harsh condition such as high temperature and dry environment of shelves surfaces at home [17-20]. It has been reported that the bacteria were greatly resistant against antimicrobial compounds [21-22]. That is why the appearance and distribution of bacteria resistant against antibiotic in the environments surrounding the hospitals posed a great challenge to the world.

Main source to spread the pathogenic bacteria from the hospital were sewage of the hospitals. The sewage water provide good growth medium which contains nutrients and other chemicals including antibiotics. The antibiotic exposure provide enough time to genes to get resistance against the antibiotic [23]. Wastewater has been a common source of antimicrobial compounds released from clinics and hospitals and these drugs are not treated [24], properly and added to the environment, which cause the resistance of bacteria living in that environment and spread to the human population [25].

Chitnis et al [26] had reported a population of bacteria in the effluent disposing from the clarifier tanks of the hospitals to the tank having treated water by physical process such as chlorination and/or flocculation. The reason could be that the sediment may have too much accumulation of bacteria along with the sediment sludge and waste and the chlorine concentration was inadequate.

Several of factors are involved to contaminate the environment by antibiotics including wastewater treatment, hospital waste, and sewage. Several sources can contribute significantly to the environmental burden of antibiotics such as hospital waste, wastewater treatment plants, sewage water, chemical pesticide usage in agriculture, inappropriate disposal of drugs [27-31]. Therefore, in result increasing resistance against antibiotic and antimicrobial compounds incidences of the appearance of resistance in clinically important pathogens such *as vancomycin-resistant enterococci* (VRE), *methicillin resistance Staphylococcus aureus* (MRSA), *Klebsiella pneumonia, Clostridium difficile,* and *Acinetobacter baumannii* have been a great manifestation of alarming situation [32-33].

The Outer layer of the earth is made of very complex inorganic minerals and valuable organic matter, which is known as soil. The microbial consortia present in the soil is greatly dependent on temperature, rainfall, pH of soil, sunlight redox potential and vegetation conditions. The pathogenic microbes may be indigenous which can penetrate by manure application, sewage, floods, contaminated water and/or animal wastes or contaminated water [34].

The top layer of the soil provides the most appropriate environment for the growth of microbes [35]. Microbes isolated from the soil provide a great source antibiotic resistant microbe (bacteria). Metabolites produced by bacteria in

plant-soil associated environment have broader spectrum activities and the microbes have adapted to coexist in different pH, human wastes, and nutrient availability.

Biotic and abiotic factors both affect the hierarchy of the microbial diversity present in the soil. Climate has been a major abiotic factor and moisture contents (humidity), temperature, pH of soil, salinity soil structure and texture play very critical role in microbial consortia and their resistance against antibiotics. Beside this, variation in climate conditions greatly influence micro-flora of soil. It is one the reason of greater variation of microbial diversity across the globe. Although the microbes are present in abundance in soil, which produce antibiotic compounds but the ecological factors, affecting the synthesis has not been clarified yet. The continuous studies are in progress to obtain new and novel antibiotics against the antibiotic resistant microbes. The present study is also a step to develop new antibiotic that could be effective against MRSA present not only in Saudi Arabia but also in UK. However, there has been no association with the distance from hospitals contain MRSA.

CONCLUSION

Findings of the present study were clear that MRSA was present in the area surround the hospitals of Saudi Arabia and UK. These results revealed that pathogenic bacteria including MRSA could be transported through various sources such as derange of hospital effluents, waste disposal, shoes of staff. The MRSA was isolated from soils round different hospitals of Saudi Arabia with greater frequency. At the first stage, the bacteria were cultured on CHRO-agar, which is a selective media for MSRA, but the selective media was used to get MSRA from the mixed culture. *Bacillus* spp. Present in soil sometime provide the indicator color change on this medium. However, the MSRA were confirmed by mauve colonies on the isolation plates that were cocci.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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