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Antibiotic Susceptibility and Heavy Metal Tolerance Pattern of *Staphylococcus epidermidis*, and curing of plasmid

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ABSTRACT

Five strains of *Staphylococcus epidermidis* isolated from burns, and susceptibility test were done to these isolates by using different antibiotics (penicillin, nitrofurantoin, ampicillin/cloxacillin, tetracycline, cephalexin, ampicillin, tobramycin, ceftazidime, imipenem, amoxicillin/clavulanic acid, cefotaxime, rifampin, cloxacillin, erythromycin, nalidixic acid and cefoxitin). The results showed that all strains were sensitive towards Nitrofurantoi n, Tetracycline, Imipenem, Rifampin and Nalidix acid. Susceptibility test to different heavy metals with different concentrations were done by using Copper, Aluminum, Iron, Cobalt, Iodine, Lanthanum and Zinc. Some isolates exhibited high sensitive to heavy metals with minimum inhibitory concentration (MIC) ranging from 1 M to 0.01M. The isolates were subjected to plasmid curing using 2% SDS for 24 hrs. After the curing, the isolates were subjected to antibiotic resistance testing and heavy metal tolerance. The isolates which lost the capacity to grow on nutrient agar plate containing iodine, zinc, ceftazidime, cefoxitin and cloxacillin contain genes on their plasmid, while the isolates which were able to grow on the other nutrient agar plates containing cefotaxime and lanthanum contain genes on their chromosome.

Keywords: *Staphylococcus epidermidis*, antibiotic, heavy metals, curing

INTRODUCTION

Staphylococci are ubiquitous, G^{+ve} that represents part of normal bacterial microflora of skin and mucosal surfaces of humans and animals. *S. aureus*, *S. epidermidis*, *S. saprophytics* are well known for implication in human health disease [1]. *S.epidermidis* has long been recognized as an important opportunistic pathogen accomiting the majority of nosocomial infections alongside *S. aureus* , however *S.epidermidis* virulence mechanisms is still limited previous have emphasized various analogies in innate agents pathogen in plant [2]. Microbial population in metal polluted environment adapt to toxic concentration of heavy metals and become metal resistant: the response of *M-ms* towards toxic heavy metals importance in view of the ability of isolated fungal and bacterial strains towards remediation of chromium and nickel evaluated by characterization [3]. The bioaccumulation of these metals effect of temperature, pH and tolerance to the heavy metals by isolated organisms. Heavy metal and inhibitory action on *M-ms* by blocking essential functional group displacing essential metal ions and modifying the active conformation of biological molecules low concentration some metal are essential for *M-ms* (Co, Cu, Zn, Ni) since they provide vital co-factor for metalloprotease and enzymes [4]. Metals are of environment interest both as limiting nutrient and as toxicant –some metals such as Zn and Mn micronutrient for microorganism have toxic effect at elevated concentration metal transport into biota involves diffusion from the bulk solution to biological surfaces binding onto organism surface and intracellular uptake or internalization through specific transported Cd and Zn chemically similar metals and can be taken up through similar transport pathways for many metals such as (Cd, Cu, Zn) [5]. Antibiotic susceptibility has also emerged as an ever increasing health hazard due to the indiscriminate use of antibiotics. This has led to severe complications in patients especially with gram negative bacterial infections as the number of drugs to combat this

category of infections are limited. Multidrug resistance (MDR) can also be caused by another mechanism of accumulation of multiple antibiotic resistance genes each coding for a single antibiotic occurring on resistance (R) plasmids [6]. Multi drug resistance organisms are posing to be a huge threat in treatment procedures due to the presence of plasmid borne mobile resistance genes that can readily spread through bacterial populations and efflux systems to counter third and even fourth generation cephalosporin [7].

MATERIALS AND METHODS

Chemicals and media

Antibiotics from Bioanalys-Turkey, Brain heart infusion broth, Mueller-Hinton agar, nutrient agar, brain heart infusion agar and nutrient broth from Biolife-Italy, copper, aluminum, iron, cobalt, iodine, lanthanum and zinc from Fluka (Switzeland), other chemicals were supplied by BDH Chemicals.

Identification of isolates

All 5 strains of *Staphylococcus epidermidis* were identified as Gram positive grape-like clusters by the Gram staining technique. Biochemical tests were conducted for all the above according to Bergey's manual of determinative bacteriology and all strains were confirmed as *Staphylococcus epidermidis*.

Antibiotic susceptibility test

The isolated strains were checked for antibiotic resistance with penicillin, nitrofurantoin, ampicillin/cloxacillin, tetracycline, cephalexin, ampicillin, tobramycin, ceftazidime, imipenem, amoxicillin/clavulanic acid, cefotaxime, rifampin, cloxacillin, erythromycin, nalidixic acid and cefoxitin. The Kirby-Bauer disk diffusion method [8, 9] was employed for antibiotic susceptibility of the 5 strains of *Staphylococcus epidermidis*. The strains were inoculated into 5 ml of nutrient broth and incubated overnight at 37°C. These pure broth cultures were then swabbed on 20 ml of nutrient agar plates each. All 16 antibiotic disks were placed on each of the swabbed plates at appropriate distances from one another and the plates were then incubated at 37°C for another 24 hrs. Zones of inhibition were obtained by measuring the diameter across the center of each zone in millimeters.

MIC determination of heavy metals by using agar dilution method

All isolates were also tested to determine the minimal inhibitory concentrations (MICs). MICs of seven metals including (Copper, Aluminum, Iron, Cobalt, Iodine, Lanthanum and Zinc) was carried out by using agar dilution method with modification as described by Narasimhulu*, et.al.,* [10], were prepared as follows: **1**- Mueller-Hinton agar was prepared in 50 ml distilled water, and approximate concentration 1M, 0,1M, 0.01M,0.001M and 0.0001 for each heavy metals, added to flask contained 50 ml Mueller-Hinton, mixed well ,and autoclaved at 121 ^{O}C for 15 minutes. **2-**After autoclaving, inoculated medium was allowed to cool (45C^{o}) and then poured into a plate. **3**- Inoculating by refreshed growth and incubated at 37C for 24 hrs. **4-** After incubation if there is growth, meaning resistance and if there is no growth meaning sensitive.

Plasmid curing by SDS

24 hours old cultures of the isolates were grown in sterile nutrient broth containing 2% SDS [11], and incubated at 37°C for 24 hrs followed by subjecting antibiotic sensitivity and heavy metals tolerance.

RESULTS AND DISCUSSION

Susceptibility to antibiotic

All the five isolates of *Staphylococcus epidermidis* that were obtained from burns, their antibiotic susceptibility are shown in Table 1. All strains were sensitive towards Nitrofurantoi n, Tetracycline, Imipenem, Rifampin and Nalidix acid. This result indicates that the strain *Staphylococcus epidermidis* exhibits resistance to wide spectrum of antibiotics, i.e.; multiple drug resistance patterns.

Table 1. Sensitivity and resistance of *S. epidermidis* for antibiotic.

Name of Antibiotic	Conc. (mcg)	Isolate1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Penicillin	10	S	R	S	R	R
Nitrofurantoi n	30	S	S	S	S	S
Ampicillin/cloxacillin	30	S	R	R	R	R
Tetracycline	30	S	S	S	S	S
Cephalexin	30	S	R	R	R	S
Tobramycin	10	S	S	S	R	R
Ampicillin	10	S	R	S	R	R
Ceftazidime	30	R	R	R	R	R
Imipenem	10	S	S	S	S	S
Amoxicillin /calvulanic acid	20/10	S	R	R	R	R
Cefotaxime	30	R	R	R	R	R
Rifampin	5	S	S	S	S	S
Cloxacillin	1	R	R	R	R	R
Erythromycin	15	S	R	R	R	R
Nalidix acid	30	S	S	S	S	S
Cefoxitin	30	R	R	R	R	R

R= resistant; S= sensitive

Since last decades spread of antibiotic resistance (AR) in bacteria, including Staphylococci has increased which represent hazard for human health. Among antibiotic resistant *staphylococci*, multidrug-resistant *S.*

aureus strains are of great public concern since resistances make treatment difficult for infections. Moreover, a number of CNS, such as several *S. epidermidis* strains, is important hospital-acquired infection agents and the 80–90% of these isolates is methicillin-resistant [12]. Antibiotic resistant bacteria are bacteria that cannot be fully inhibited or killed by an antibiotic. The antibiotic may have worked effectively before the resistance occurred. Bacteria become resistant to antibiotics by adapting their structure or function in some way that prevents them from being killed by the antibiotic. This mechanism might happen in several ways; bacteria can neutralize the antibiotic before it has an effect, bacteria may be able to pump the antibiotic out, bacteria may be able to change the site (receptor) where the antibiotic normally works and bacteria can mutate and transfer genetic material that codes for resistance to other bacteria. The resistant bacteria that survive the effect of the antibiotic are able to multiply, spread to others and cause further infections in the family, community, and/or health care setting. In turn, these infections are more resistant to another round of the same antibiotic. To have a better understanding of antibiotic resistance, Table 1 lists common bacteria that have become highly resistant, associated antibiotics with reduced activity, and antibiotics that may be appropriate for treatment of that resistant bacteria [18].

Susceptibility to heavy metals using Agar dilution method

In present study, the effects of seven heavy metals on *S. epidermidis* isolates were investigated by using MIC in different molar concentration, the heavy metals were used copper, aluminum, iron, cobalt, iodine, lanthanum and zinc. Results revealed that five isolates were sensitive to copper and cobalt at concentration 1M, 0.1M and 0.01M, while five isolates were sensitive to aluminum at concentration 1M and 0.1 M. Five isolates were resistant to iodine, lanthanum, and zinc, while four isolates were resistant to iron except one isolate sensitive to iron at concentration 1M Table 2.

Table 2. Sensitivity and resistance of *S. epidermidis* for heavy metals.

Isolate	Isolate 1					Isolate 2					Isolate 3					Isolate 4					Isolate 5				
Metals conc.(M)	1	0.1	0.01	0.001	0.0001	1	0.1	0.01	0.001	0.0001	1	0.1	0.01	0.001	0.0001	1	0.1	0.01	0.001	0.0001	1	0.1	0.01	0.001	0.0001
Copper	0	0	0	R	R	0	0	0	R	R	0	0	0	R	R	0	0	0	R	R	0	0	0	R	R
Aluminum	0	0	R	R	R	0	0	0	R	R	0	0	R	R	R	0	0	R	R	R	0	0	R	R	R
Iron	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0	0	R	R	R	R	R	R	R	R
Cobalt	0	0	0	R	R	0	0	0	R	R	0	0	0	R	R	0	0	0	R	R	0	0	0	R	R
Iodine	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Lanthanum	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Zinc	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

R= resistant, 0= no growth or sensitive

The interpretation of these results may be due to the fact that *S. epidermidis* has many mechanisms for heavy metals resistance. Firstly, the accumulation of specific ions can be diminished, not by interference with uptake but by active extrusion of the heavy metal ions from the cells, this mechanism is specific only for *staphylococci* spp., Secondly, cations can be segregated into complex compounds by thiol- containing molecules and then ejected from cell, thirdly, some metal ions may be reduced to a less toxic oxidative state by the complex enzymes and special oxidation mechanisms in the cells. Finally, for many metals resistance and homoeostasis is a combination of two or three of the mentioned basic mechanisms that is the case which *S. epidermidis* succeeds [13]. However, most of the isolates in the present study showed multiple tolerances to both heavy metals and antibiotics. The microbial resistance to heavy metal is attributed to a variety of detoxifying mechanisms developed by resistant microorganisms such as complexation by exopolysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another [14]. Prasad *et al*.,[15], found that all isolates were sensitive to heavy metals (Cd2+, Ag+, Ar2+, Co2+, Ni2+, Hg2+, and Pb2+) at concentration 0.1M, and most of them were resistant to heavy metals at concentration (0.0001M). The interaction between heavy metals and antibiotic resistance are of three types: heavy metals interaction with antibiotic compounds, heavy metals interaction with antibiotic resistance genes or even their products and heavy metal interaction with bacterial properties like conjugation [16], of cations heavy metals complex with antibiotics.

Plasmid curing by SDS

The isolates treated with 2% SDS, showed significant change in their tolerance of heavy metal and antibiotic sensitivity, indicating that 2% SDS could be used as a plasmid curing agent. 24 hours old cultures of isolates were subjected to plasmid curing using 2% SDS for 24 hrs. After the curing, the isolates were subjected to antibiotic resistance testing and heavy metal tolerance. After comparing the results of the antibiotic resistance testing and the heavy metal tolerance studies (performed before the plasmid curing), the five isolates lost the capacity to grow on nutrient agar plate containing ceftazidime, cefoxitin, cloxacillin**,** iodine and zinc, while the isolates were able to grow on the other

nutrient agar plates containing cefotaxime and lanthanum. This indicated that isolates which lost the capacity to grow on nutrient agar plate containing iodine, zinc, ceftazidime, cefoxitin and cloxacillin contain genes on their plasmid, while the isolates which were able to grow on the other nutrient agar plates containing cefotaxime and lanthanum contain genes on their chromosome. Durve, *et.al.* [17], found that Ethidium bromide (100 mcg/ ml) was a much efficient agent for curing the plasmid compare with 2% SDS.

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