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Susceptibility of Some *Staphylococcus aureus* Isolates to Cadmium, Mercury and Ultraviolet Radiation

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Abstract. Some *Staphylococcus aureus* isolates were studied with respect to their susceptibility to UV radiation, mercury and cadmium. The resistances to mercury and cadmium were mediated by 38.5 and 4.2 Mdal plasmids, respectively. The isolates were inactivated exponentially by UV radiation with some resistant subpopulation at higher UV doses. The pigmented isolates were more resistant than the non-pigmented ones, moreover, they have shown better photoreactivation. There was no correlation between heavy metal and UV resistances, further, the plasmids had no effect on their UV susceptibility.

Introduction

Heavy metals are generally toxic for both gram-positive and gram-negative bacteria [1, pp. 222-237] and some, such as mercury and cadmium, are considered to be highly toxic [2;3]. Several different mechanisms of bactreial resistance to heavy metals have been reported [4-6] and found to be plasmid encoded. Moreover, there is a close correlation between heavy metal resistance and antibiotic resistance *i.e.* antibiotic resistance plasmids, or chromosomal genes (vary geographically) of a wide variety of bacteria, including *Staphylococcus aureus,* contain genes conferring resistance to heavy-metal cations and anions including mercury and cadmium [7;8].

Germicidal range of UV radiation does not produce undesirable by-products and is effective in activating a variety of microorganisms [9;10]. Further, the range of UV dose necessary to disinfect pathogens is narrower than it is for chlorine disinfection [9], therefore, the UV is more effective than chlorination in killing pathogens. Bacteria, however, vary with respect to their UV sensitivity according to strain, sporulation, growth medium, stage of growth, and influences of the plating medium on repair of sublethal damage [11-14]. Chang *et al.* [9] found that *Escherichia coli, Shigella sonnei, Salmonella typhi,* and *S. aureus* exhibited similar resistances to UV light and required about the same dose for 3 log units of inactivation. *Streptococcus faecalis,* however, required a 1.4 times higher dose for inactivation. Pyrimidine

dimers, which are formed in the DNA as a result of UV irradiation, could lead to mutagenic change or cell death. Several repair pathways exist for the repair of UV-induced DNA damage, including photoreactivation, excision repair, recombinational repair, and inducible error-prone repair [15, pp. 22-40; 16]. As plasmids could mediate heavy metal resistance, they can also mediate the sensitivity of some bacteria to UV light [17]. The objective of this study, therefore, was to evaluate the sensitivity of some *S. aureus* isolates with different plasmid contents to mercury, cadmium and UV light.

Materials and Methods

Bacterial isolates

Several environmental isolates of *Staphylococcus aureus,* obtained and identified by this laboratory, were used in this study. Three of these isolates were pigmented (Isolates 29, 55 and 76), whereas three others were non-pigmented (Isolates 3,21 and 21A). Isolate 21A was obtained by plating isolate 21 on brain heart infusin agar plates (Difco) containing 8μg/ml gentian violet. The isolates were grown on brain heart infusion agar slants at 37°C and stored in the refrigerator with monthly transfers. Stock cultures were kept as cell suspensions at –20°C in 30% glycerol – 1% peptone.

Effects of cadmium and mercury on the *S. aureus* isolates

The susceptibility to cadmium and mercury was estimated by two ways:

1) Plate diffusion method

The plate diffusion method described by Abbas and Edwards [6] was used for the qualitative estimation of the tolerance of the *S. aureus* isolates listed above to mercury and cadmium as follows:
A trough, 0.2 by 90 mm, was cut into brain heart infusion agar contained in a square dish (10 by 10 cm). Into the trough was added 0.2 ml of stock solution of 0.25 M $HgCl_2$ or 0.89 M $CdCl_2$. The metal diffusion was allowed for 24 hr at 30°C and the isolates were then streaked at right angles to the trough. The distance of the growth inhibition (in millimeters) was determined after 24 hr incubation at 30°C.

2) Minimum inhibitory concentration (MIC)

The MIC was determined as described by Cooskey *et al.* [4]. In brief, isolates were grown on brain heart infusion agar plates containing different concentrations of $CdCl_2$ or $HgCl_2$. The plates were incubated at 37°C for 24 hr. The heavy metal concentration which did not have confluent grwoth was considered as the MIC.

Sensitivity of the *S. aureus* isolates to UV light

Isolates were grown to mid exponential phase in brain heart infusion broth at 37°C. Cells were centrifuged at 10,000 × g for 5 min at 4°C, washed with and suspended in 0.85% NaCl to a concentration of 2×10^6 cells per ml. Aliquots (10 ml) were UV irradiated in open glass petri plates for various times with a germicidal lamp (Ultra-Violet Products Inc., San Gabriel, Calif.) which emits mainly at 254 nm with an irradiation intensity of 580 $\mu W/cm^2$ at 15 cm. Unwanted photoreactivation was avoided by working in a darkroom with a red light to prevent photoreactivation. Half of each UV-irradiated cell suspension was transferred to a sterile test tube kept in the dark in a beaker of ice until the completion of the experiment. The other half was transfered to a sterile test tube and light exposed 60 min in a beaker of ice placed near a 500-watt bulb. Viable cells counts were determined by plating 0.1 ml aliquots of the appropriate dilutions on brain heart infusion agar plates and incubating the plates overnight at 37°C. The surviving fractions were plotted as a function of the UV dose.

Plasmid analysis

Isolates were screened for plasmid content by the method of Holmes and Quigley [18] modified for *S. aureus* [19]. In brief, cells were grown overnight on brain heart infusion agar and were suspended in 10 ml of saline (0.5% NaCl) to an optical density of 1.0 at 620 nm. The cells were transferred to 1.5 ml Eppendorf tubes and harvested by centrifugation. The pellets were suspended in 0.4 ml of STET buffer (8.0% sucrose, 5.0% Triton X-100, 50 mM EDTA, 50 mM Tris; pH 8.0) and transferred to new 1.5 ml Eppendorf tubes. After the addition of 12 μl of lysostaphin (Sigma Chemical Co.; 10 mg/ml in 0.05 M Tris [pH 7.5] – 0.145 NaCl), the tubes were placed in boiling water for 50 seconds and immediately centrifuged (15,000 × g) for 8 min. The supernatants were transferred to new centrifuge tubes and the nucleic acid precipitated by the addition of 2 volumes of 95% ethanol. After centrifuging for 5 min., the precipitated DNA were suspended in 20 μl of TES buffer (30 mM Tris [pH 8.0], 5 mM EDTA, 50 mM NaCl). Ten microliters of each sample and 5 μl of running dye were electrophorsed in 0.7% agarose gels in tris-borate buffer at 100 V for 90 min, followed by ethidium bromide staining and photography under UV light.

Results

Effect of cadmium and mercury on the *S. aureus* isolates

The effects of cadmium and mercury on some *S. aureus* isolates are shown in Table 1. The distance of growth inhibition [%] and the minimum inhibitory concentration results agree with each other, that is, isolates 3 and 76 were resistant to cadmium only, isolates 29 and 55 are resistant to mercury only, whereas, isolate 21 and its mutant (21A) are resistant to both or sensitive to both, respectively.

Table 1. Cadmium and mercury tolerance of selected *S. aureus* isolates assessed by the minimal inhibitory concentration (MIC) and plate diffusion methods

Isolate no.	Minimal inhibitory concentration (mM)		Distance of growth inhibition (%)	
	Cd^{2+}	Hg^{2+}	Cd^{2+}	Hg^{2+}
3	1.2	0.04	43.3	71.1
21	1.2	0.08	42.2	34.4
29	0.6	0.08	78.9	36.7
55	0.8	0.08	76.7	32.2
76	1.2	0.04	43.3	68.9
21A	0.6	0.04	77.8	72.2

Sensitivity of the *S. aureus* isolates to UV light

UV dose-survival curves of log-phase cells of isolates 3, 21 and the mutant 21A (not pigmented) and isolates 29, 55 and 76 (pigmented) are compared (Fig. 1). Both non-pigmented and pigmented isolates were inactivated rapidly and showed a leveling off at higher UV doses. Further, the UV irradiated cells which were subsequently exposed to visible light showed higher recoveries than UV-irradiated cells kept in the dark (photoreactivation). In addition, the pigmented isolates appeared to be more resistant to UV light than the non-pigmented isolates.

Plasmid analysis

Three plasmids of different sizes were detected (Fig. 2); 38.5 Mdal (isolates 21, 29 and 55), 21 Mdal (isolates 29 and 55) and 4.2 Mdal (isolates 3, 21 and 76). The mutant 21A was cured of the two plasmids found in isolate 21. The 21 Mdal plasmid did not appear to play any role in these studies.

Discussion

S. aureus is a hearty organism present in a wide variety of environments. It has long been considered as an important hospital and community pathogen [20], which vary from country to country with respet to heavy metal and UV radiation resistances [1;8;9;12]. This study, therefore, was performed to determine if any correlation could be observed between the heavy metal and UV resistances and whether these resistances could be related to plasmid contents of the cells.

The results observed here for cadmium and mercury resistances are: resistance to both mercury and cadmium by isolate 21 which has 38.5 and 4.2 Mdal plasmids; resistance to cadmium but not mercury by isolates 3 and 76 which have 4.2 Mdal plas-

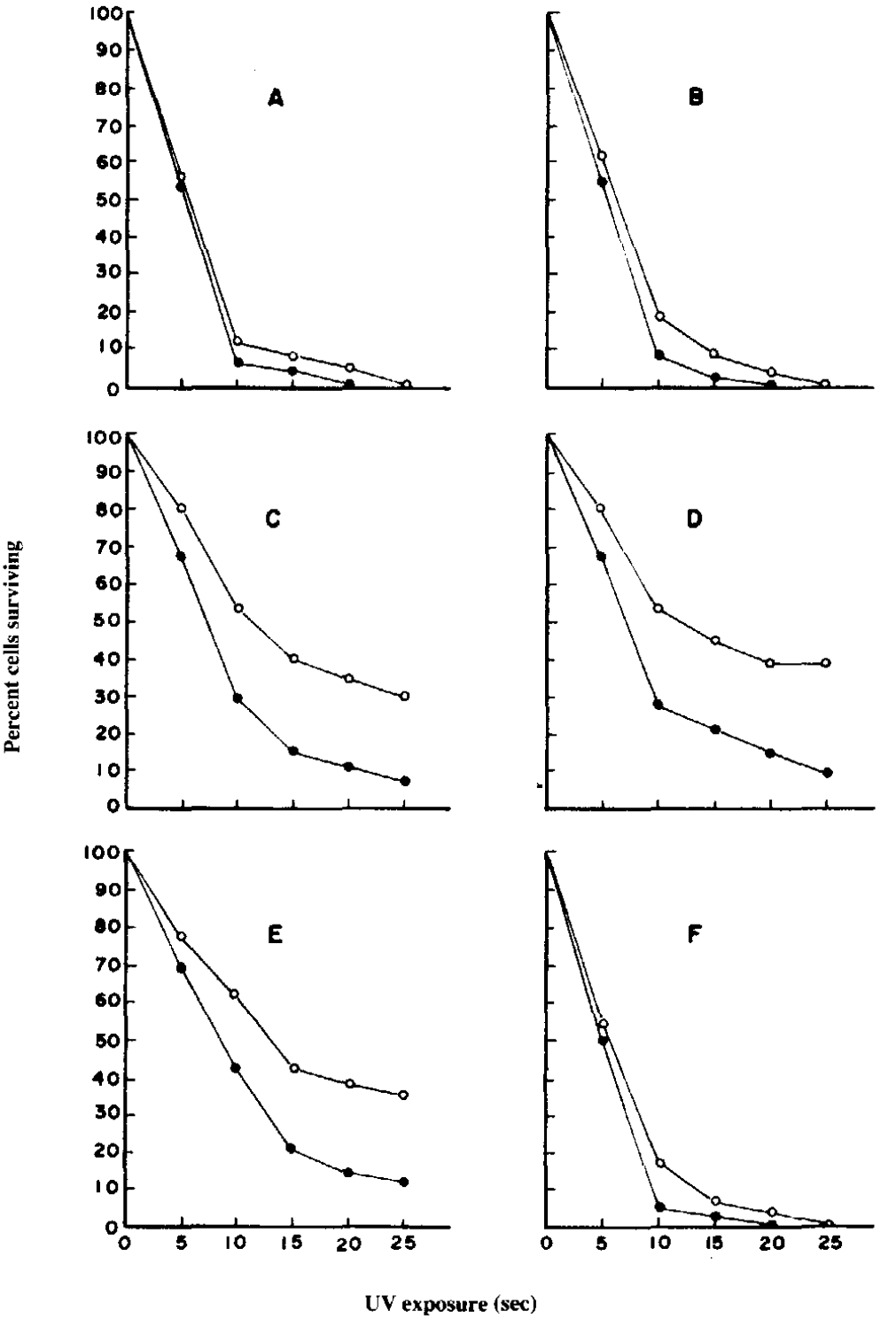

Fig. 1. UV inactivation and photoreactivation of *S. aureus* isolates. Cells were irradiated with various doses of UV and either diluted and plated in the dark (•) or exposed to photoreactivating light for 1 hr and plated (o). The isolates are: (A) 3; (B) 21; (C) 29; (D) 55; (E) 76; (F) 21A.

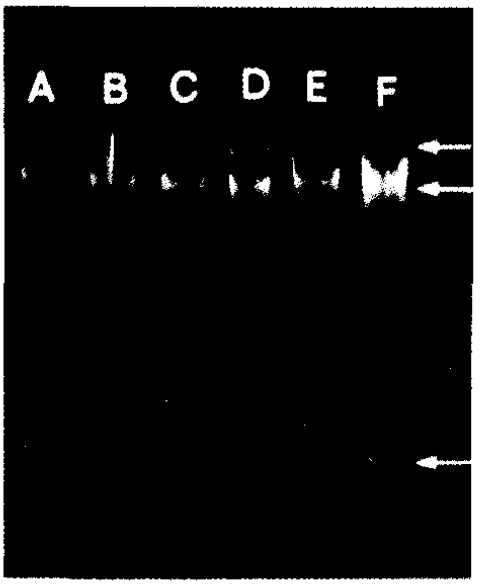

Fig. 2. Plasmid content of *S. aureus* isolates. The lanes: A, B, C, D, E, and F are for the isolates 3, 21, 29, 55, 76 and 21 A, respectively. The arrows to the right of lane F indicate from top to bottom the position of the 38.5 Mdal plasmid, Chromosomal DNA and 4.2 Mdal plasmid.

mid; loss of resistance to both cadmium and mercury by isolate 21A which was cured from both 38.5 and 4.2 Mdal plasmids. These results suggested that the 38.5 and 4.2 Mdal plasmids somehow appear to be associated with mercury and cadmium resistances, respectively, whereas the 21 Mdal plasmid does not appear to be associated with either mercury or cadmium resistances in these studies. The above 38.5 and 4.2 Mdal plasmids vary in size from those plasmids of *S. aureus* associated with heavy metal resistances reported by other authors [1;8;20]. Further, these results show that the cadmium and mercury resistances reported in this paper are plasmid associated and not due to chromosomal DNA [8].

Plasmids containing heavy metal resistance genes of many environmental bacteria have been shown to be indicators of environmental pollution by these two heavy metals [1;3]; further, they have been shown to code for antibiotic resistance [8;21;22]. The heavy metal resistance of *S. aureus* isolates under study, therefore, would be of great concern. To date, much have been published on the inactivation of microorganisms by UV light [9;10;12;13]. The results obtained for the isolates under study showed some flattening of curves at higher UV doses, which could be indicative of UV resistant subpopulations. These resistant subpopulations might reduce the value of UV light as an inactivation tool for this organisms. The increased survival of UV irradiated cells (being more evident for pigmented cells than the non-pigmented cells) exposed to visible light over the UV-irradiated cells kept in the dark, is indicative of repair of UV-indiced damage for both isolates by photoreactivation.

The differences among the *S. aureus* isolates in regard to UV sensitivity may relate to their pigmentation, since pigmented isolates were more resistant. A number of bacterial plasmids have been described which alter the sensitivity of the organism towards UV light. Some plasmids confer to the host cells reduced susceptibilities to killing by UV radiation [23;24], other plasmids render their host more sensitive to UV [17], and still others have no effect on UV sensitivity [16]. The studies reported in this paper showed that the plasmids observed did not alter the sensitivity of the *S. aureus* to UV radiaiton. Moreover, no correlation is apparent between cadmium and mercury resistances and UV resistance of this organism.

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حساسية بعض عزلات البكتيريا ستافيلوكوكس أوريس للكادميوم والزئبق والأشعة فوق البنفسجية

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(سُلِّمَ في ٧ ذو القعدة ١٤١١هـ، وقُبل للنشر في ٢ ربيع الآخر ١٤١٣هـ).

ملخص البحث. لقد دُرِسَتْ حساسية البكتيريا ستافيلوكوكس أوريس لكلٍّ من الأشعة فوق البنفسجية والزئبق والكادميوم. المعلومات الوراثية لمقاومتي كلًّا من الزئبق والكادميوم منقولة على البلازميدين ٣٨,٥ و٤,٢ ميجادالتون، على التوالي. لقد ثبطت العزلات طرديًّا بواسطة الأشعة فوق البنفسجية مع وجود جيل ثانوي مقاوم عند الجرعات العالية. العزلات الملونة أكثر مقاومة للأشعة فوق البنفسجية من العزلات غير الملونة كما أنها نشطت ضوئيًّا بصورة أفضل. لا توجد علاقة بين مقاومة كلًّا من الأملاح الثقيلة والأشعة فوق البنفسجية كما أن البلاميدات لا تؤثر على حساسية هذه البكتريا للأشعة فوق البنفسجية.