STATISTICAL ANALYSIS OF AIR-BORNE BACTERIA ISOLATED FROM DIFFERENT SITES OF KARACHI UNIVERSITY

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SUMMARY: Air-borne bacteria were isolated from different sites of Karachi University. These isolates were purified and checked against different antibiotics like ampicillin, kanamycin, tetracycline and streptomycin and against metal salts like Copper sulphate, Cadmium chloride, Cobalt chloride, Nickel sulphate and Mercury chloride. Most of these isolates were found to be highly resistant to the tested metals and antibiotics. More samples are being collected to have a proper idea of environmental status of the Karachi metropolis. These studies will provide an idea of environmental conditions and can be used for its monitoring. Statistical analysis has showed that there was no difference in the three environments. Correlation was also observed for metal and antibiotic resistance in the organisms of experimental area.

Key words: Air-borne bacteria, resistance, pigmentation.

INTRODUCTION

The biological diversity and environment are hot topics of the day. Looking at it from a wider perspective, one may say that biological diversity is also an integral part of environment and that the two together make it possible for humanity to survive.

It has become a big challenge for humanity as to how they can industrially develop to meet the growing demands of the humans and at the same time adopt safer and pollution free technologies.

Bacteria can combat heavy metal stresses in the environment and they detoxify the adverse effect by different inherent mechanisms. Bacterial strains showing multiple resistance to different antibiotics were first isolated in Japan during 1952-59. Such strains have since been reported from other parts of the world uncovering an increase in the incidence of multiple antibiotic resistance among gram-negative bacteria. The responsible genes in most of the organisms are present on extrachromosomal material i.e, plasmid. Since metal resistance and antibiotic resistance genes are often carried by the same plasmid (6, 7, 11), the frequent correlation of metal and antibiotic resistance in bacteria from polluted environments exists. Metal tolerance might have been developed through physiological adaptations (5, 1), or by selection of types of bacteria that are inherently metal resistant (11).

The present study was carried out to obtain a clear understanding of environmental status by studying air-borne bacterial strains isolated from different sites of Karachi University.

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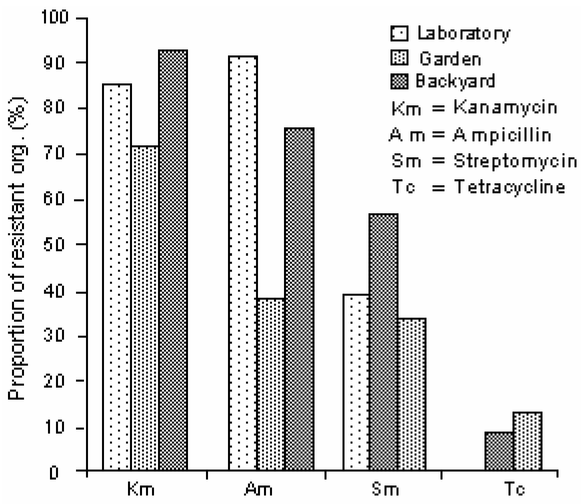
Figure 1: Antibiotic resistance of the bacterial isolates among sampling areas.



MATERIALS AND METHODS

Sample collection, isolation and purification

Samples were collected from three different sites around the Centre for Molecular Genetics (CMG) laboratories, University of Karachi. The three sampling sites were linked to each other, samples were collected from these sites so as to compare them with respect to pollution. The sampling sites were CMG laboratories, CMG garden and CMG backyard.

Samples were collected by Settle plate method (9). Prepared nutrient agar plates were opened in the above mentioned locations from 1-2 m above ground level for about 30 minutes. Samples were collected at the same time and day so as to provide same conditions for comparison. The samples from CMG laboratories, garden and backyard were labelled as L, G, and B respectively. The three sets of plates were incubated at 37°C and growth was observed after 24 hours. Total 57 cultures were purified from three samples. 24 were from sample of CMG laboratories and were coded as L1, L2, L3……..L24, from garden 18 isolates were purified and labelled as G1, G2, G3....G18. While from backyard 15 isolates were purified and were coded as B1, B2, B3....B15. These isolates were preserved in Nutrient agar and Tris minimal agar (4) stabs.

Assessment of heavy metal and antibiotics resistance

Metal and antibiotic resistance was studied according to the standard procedure using Tris minimal medium. Metal salts used were chromium chloride, copper sulphate, cobalt chloride, cadmium chloride, nickel sulphate whereas antibiotics tested included ampicillin, kanamycin, streptomycin and tetracycline.

Statictical analysis

Statistical analysis of three sampling areas was done, this analysis consisted of percentage comparison, correlation and Krukal-Wallis test (2) with respect to their heavy metal and antibiotic resistance of bacteria.

RESULTS

A total of 57 bacterial isolates were obtained from three different locations of CMG of Karachi University. Most of the bacterial isolates of the sampling sites showed resistance to different metal salts and antibiotics. The isolates of sampling sites showed approximately similar resistance against cadmium salt with respect to three sampling sites, where as the Ni^{+2}, Cu^{+2}, Co^{+2}, and Hg^{+2} showed different patterns (Figure 2). Results of antibiotic resistance showed that the Laboratory strains were highly resistant against ampicillin, kanamycin and streptomycin (Figure 1). There rarely was a difference observed among the bacterial isolates of three different sampling areas against Streptomycin and there was no resistance against tetracycline. Pigmentation was assessed initially on nutrient agar and subsequently on tris minimal agar, obvious differences in pigmentation were observed on the two media (Table 1). The difference was observed between the sampling sites with respect to metal resistance and antibiotic resistance. Result showed that we had insuf-

Table 1: Percentage chart for pigmentation

Nutrient agar								
	Yellow	Orange	Pink	Light Pink	Creamy White	White	Pig. Col. %	White Col. %
Laboratory	4	12.5	12.5	NF	45.8	25	75	25
Garden	33.3	5.5	22.2	NF	22.2	16.6	83	16.6
Backyard	33.3	26.6	NF	13.3	13.3	13.3	86.7	13.3
Total pig. (%)	21.05	14.03	12.28	3.5	29.82	19.29	80.7	19.3

Tris agar								
	Yellow	Orange	Pink	Light Pink	Creamy White	White	Pig. Col. %	White Col. %
Laboratory	NF	-	8.3	8.3	NF	83.3	16.7	83.3
Garden	16.6	5.5	NF	22.2	NF	55.5	44.5	55.5
Backyard	NF	20	NF	NF	20	60	40	60
Total pig. (%)	5	7	10	3	5	68.4	31.5	68.4

NF = Not found

ficient evidence to reject the null hypothesis that there was no difference in the three environments (h>5.991, Table 3) of sampling sites. Correlation analysis was also performed to check whether there was any correlation between the metal and antibiotic resistance in the whole sampling area. For correlation analysis total number of colonies were taken which observed on different concentrations of antibiotics and metal salts (Table 2) in the experimental area. In the experimental area correlation were good (Figure 3). 62% of the variation in the values of the antibiotics was accounted for by a linear relationship with metals in whole experimental area. Significance of correlation was checked by t-test (H_o: p = 0, H_A: p ≠ 0) for total number of colonies resistant to antibiotics and metals on different concentrations, result showed that there was very strong correlation (t= 4.05, $t_{0.01(2), 10}$=3.169, reject H_o, P < 0.01) between antibiotics and metals.

DISCUSSION

Presence of pollutants in air is causing great danger for all living organisms. Bacteria are in the front line, coping with pollutants in the environment. Pigmentation is known to protect the cells from damage due to sunlight (10). Difference in pigmentation was observed on the two media (Table 1). Colour changes were observed with increased concentration of antibiotics and metals on tris minimal agar. In experimental areas most of the bacteria were pigment producers. 80.7% of total number of organisms were pigment producers on nutrient agar while 19.29% were the organisms which produced white colonies. The percentage of pigmentation was reduced on tris minimal agar, out of total number of colonies 31.5% were pigment producers while 68.4% produced white colonies. For the comparison of pollutants in the sampling area resistance was checked for heavy metal and antibiotics. Highest resist-

Table 2: Number of colonies on different concentrations of antibiotics and metals.

No of tested A/M	AL	AG	AB	ML	MG	MB
1	63	18	33	34	35	33
2	59	32	40	44	36	21
3	26	21	16	23	27	24
4	0	4	0	11	9	7

A = Antibiotics M = Metal salts

ance was observed against Ni^{+2} salt (Figure 2). Ni^{+2} is normally required in trace by bacteria and it is component of many enzymes but at 100 μM concentration Ni^{+2} salt impair or suppress growth (3). Isolates of garden which showed Ni^{+2} resistance might be required Ni^{+2} as a trace element, least resistance was observed against Cd^{+2} and Hg^{+2} which are non essential and highly toxic metals for microorganisms where Ni^{+2}, Cu^{+2} and Co^{+2} are required in trace amounts as micro-nutrients. Antibiotic sensitivity was checked against (Am, Km, Sm, Tc). Isolates showed a high degree of resistance against Am and Km (Figure 1). All isolates showed multiple antibiotic resistance. Highest degree of resistance was observed in laboratory isolates for tested antibiotics, which may be due to excessive use of antibiotics in the CMG laboratories. They showed highest resistance against Am (Figure 1). The reason for this resistance could be that in CMG laboratories antibiotics are constantly used for research. There was a possibility of the atmospheric bacteria becoming antibiotic resistant and in some cases antibiotic resistance was present with metal resistance gene, in this way metal resistance could have also been transferred to air borne/atmospheric bacteria.

Table 3: Kruskal-Wallis Test.

	Heavy metals	Antibiotics
Critical region	$h > X^2_{0.05} = 5.991$	$h > X^2_{0.05} = 5.991$
Computed h	0.87	0.93

The differences observed in CMG Laboratories, CMG Garden and CMG Backyard (the sampling areas

Figure 2: Metal resistance of the bacterial isolates among sampling areas.

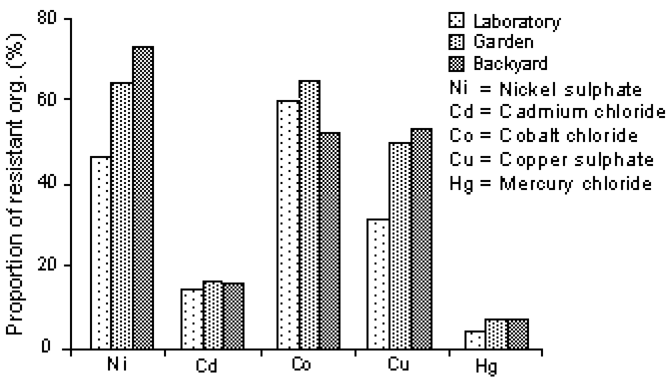
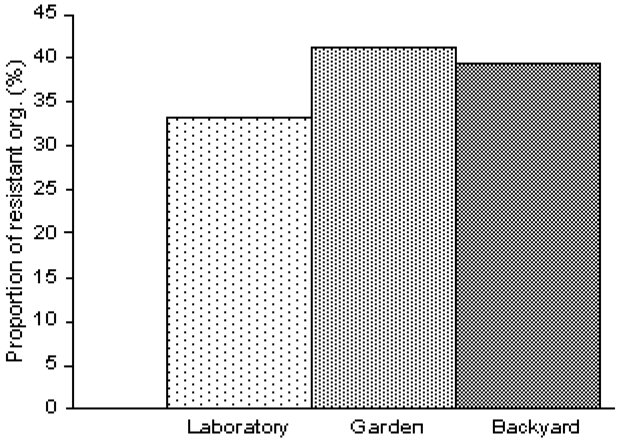


Figure 3: Plotting between total resistant colonies for metals and antibiotics.



among antibiotic resistance and metal resistance) were not significant (Table 3). This may be due to the small sample size or due to connection of three environments through windows and doors which are frequently used.

Strong correlation was proven by the t-test between the antibiotics utilized and metal salt resistance in the isolates (Figure 3). This relation could have been due to excessive work done using antibiotics in the CMG laboratories on different kinds of bacteries isolated from various polluted sites. If these bacteries of the laboratory had developed plasmid mediated antibiotic resistance there the possibility arises of transferring these plasmids to the atmospheric bacteria.

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