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Comparative Study of Air-borne Bacteria Isolated from Karachi University

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Abstract: A total of 57 airborne bacteria were isolated from different locations of Centre for Molecular Genetics, University of Karachi. 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, Nickle sulphate and Mercury chloride. Most of the isolates were found to be highly resistant to the tested metals and antibiotics. 80.7% of the total organisms were pigment producers. These studies provide an idea of environmental status and can be used for its monitoring. Statistical analysis showed that no difference in three sampling sites.

Key words: Resistance, environment and pigmentation

Introduction

The impact of various forms of environmental pollution is being increasingly felt all over the world. Accordingly agencies in Pakistan, like most other countries of the third world, have become increasingly interested in environmental problems for some past years. The warning signals are already very clear in several instances such as global warming, ozone holes, industrial pollution etc.

The pollution changes the environment and makes it difficult to often impossible for the biological diversity to survive as the entire ecological system gets damaged. It has become a big challenge for humanity as to how they can go, for the industrial development to meet the growing demands and at the same time adopt safer and pollution free technologies.

Bacteria can combat heavy metal and antibiotic stresses in the environment, they also have the ability to detoxify the adverse effects of these pollutants. Bacterial strains showing multiple resistance to antibiotics and heavy metals have been isolated from many parts of the world (Raihan *et al.,* 1995). The vast majority of micro-organisms are used for monitoring pollution. Presence of bacteria showing high resistance to heavy metals or antibiotics is an indicator of prevalence of such pollutants in the environment where these bacteria are surviving. The responsible gene in most of the organisms is present on extrachromosomal material i.e, plasmid. Since metal resistance and antibiotic resistance genes are often carried on the same plasmid (Nakara *et al*., 1977), correlation of metal and antibiotic resistance frequently found in bacteria isolated from polluted environments. Metal tolerance might have been developed through physiological adaptations (Ahmed *et al.,* 2000), or by selection of types of bacteria that are inherently metal resistant (Summers, 1978). Bacteria are often used as indicators of environmental pollution.

The present study was carried out to have a proper idea of environmental status of air borne bacterial strains isolated from different sites of Karachi University.

Materials and Methods

Media used: Tris minimal media and nutrient broth were used. All the constituents were bought from Merck (Germany) while Nutrient broth was of Oxoid (U.K). Metal salts were also from Merck.

Sample collection sites (Experimental area): Samples were collected from three different sites of CMG lab and location around the Centre for Molecular Genetics labs Department of Genetics, University of Karachi. The duration of the project was one year. 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:

i) CMG labs
ii) CMG garden
iii) CMG backyard

Collection, isolation and purification of samples: Samples were collected by Settle plate method (Russell *et al.,* 1984). 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 condition for comparison.

The samples from CMG labs, 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 h.

Total 57 cultures were purified from three samples. Twenty four cultures were from sample of CMG labs they were coded as L1, L2, L3.....L24 and from garden 18 isolates were purified they were coded as G1, G2, G3.... G18. While from backyard 15 isolates were purified and they were coded as B1, B2, B3....B15. These isolates were preserved in Nutrient agar and Tris minimal agar (Mergeay *et al.,* 1985) stabs.

Assessment of heavy metal and antibiotic: Metal and antibiotic resistance was studied according to the standard procedure using Tris minimal medium. Metal salts used included Chromium chloride, Copper sulphate, Cobalt chloride and Nickle sulphate whereas antibiotics tested included Ampicillin, Kanamycin, Streptomycin and Tetracycline. Their stock solutions were prepared as described in Maniatis *et al.* (1982).

Statistical analysis: Statistical analysis of three sampling areas were done, this analysis consisted of percentage comparison, correlation and Krukal- Wallis test (Conover, 1980) with respect to their heavy metal and antibiotic resistance of bacteria.

Results

From three different locations of Centre for Molecular Genetics of Karachi University, total of 57 bacterial isolates were obtained. These isolates were purified and checked for their ability to resist to different antibiotics and metals. 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 (Fig. 1). Isolates from the garden site showed high degree of resistance against metals. Results of antibiotic resistance showed that the Lab isolates were highly resistant against ampicillin, kanamycin and streptomycin and were sensitive against tetracycline (Fig. 2). 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). Increased concentration of antibiotics and metals salts in both the media inhibited pigmentation of bacterial strains and most of the strains became white. The difference was observed between the sampling sites with respect to metal resistance and antibiotic resistance, with the highest resistant of metal found at the Lab. These differences were analysed by Kruskal-Wallis test to find out whether the three environments with respect to their metal and antibiotic resistance were same or different. Result showed that we had insufficient evidence to reject the null hypothesis and that there was no difference in the three environments ($h > 5.991$, Table 2) of the sampling sites.

Discussion

Wide ranges of airborne bacteria are present in the atmosphere. Most of the research of airborne bacteria have been conducted about aerosolization of bacteria and effect of osmoprotectant and resuscitation agents (Marthi *et al.,* 1991; Marthi and Lighthart, 1990). 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. Shaffer and Lighart (1997), had carried out a study on microbial air pollution. Their study surveyed the atmospheric bacteria at four different location of Oregon City, on the basis of quantity and type of bacteria, pigmentation and particle size. This study was carried out to have an idea of bacterial diversity in our environment so that this could later on serves as a basis for developing database of the microbial load and to have a preliminary idea of the environmental status. Pigmentation is known to protect the cells from damage due to sunlight (Rheinheimer, 1974). Pigmentation was assessed on nutrient agar and on tris minimal agar, obvious differences in pigmentation were observed on the two media (Table 1). Colour changes were observed with increased concentration of antibiotics and metals on tris minimal agar. Among the total isolates 80.7% of organisms were pigment producers on nutrient agar while 19.29% were the organisms which produced white colonies. The percentage of pigmentation reduced on tris minimal agar out of total number of colonies 31.5% were pigment producers while 68.4% produced white colonies. Shaffer and Lighart (1997) found that 15-64% of the total

Table 1: Percentage Chart for pigmentation

	Yellow	Orange	Pink	Light Pink	Creamy White	White	Pig. Col (%)	White Col. (%)
Nutrient agar								
Lab	4.0	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
Black Yard	33.3	26.6	NF	13.3	13.3	13.3	86.7	13.3
Total % of pig.	21.05	14.03	12.28	3.5	28.82	19.29	80.7	19.3
Tris agar								
Lab	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
Black Yard	NF	20	NF	NF	20	60	40	60
Total % of pig.	5	7	10	3	5	68.4	31.5	68.4

NF= Not found

Table 2: 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

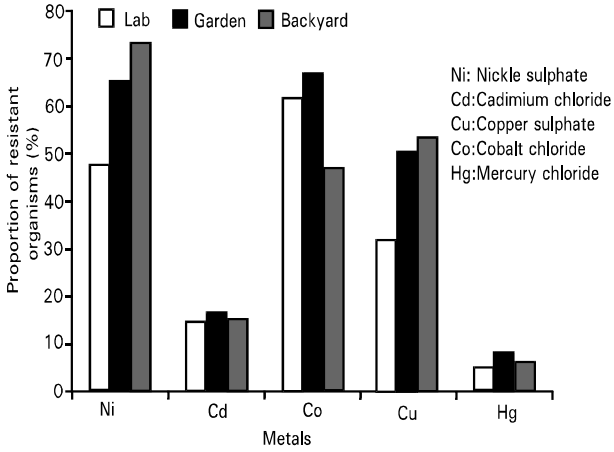

Fig. 1: Metal resistance of the bacterial isolates among sampling areas

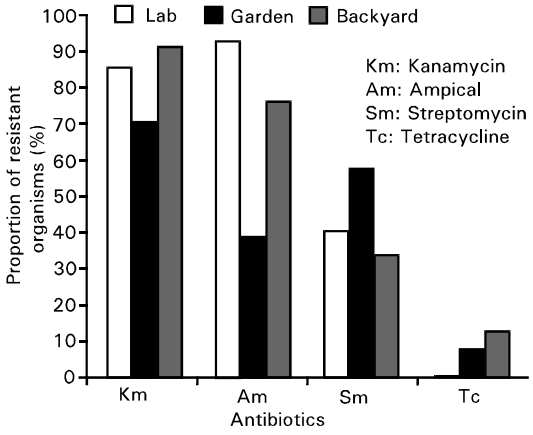

Fig. 2: Antibiotic resistance of the bacterial isolates among sampling areas

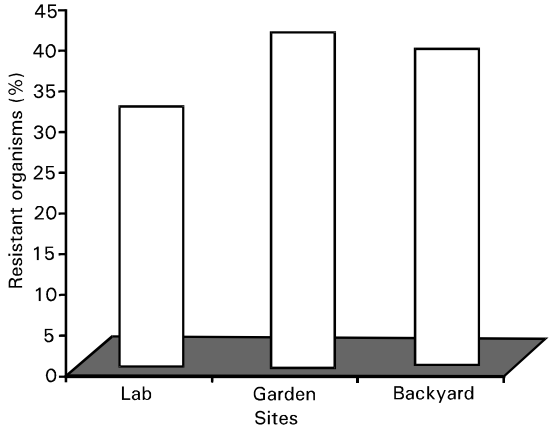

Fig.3: Comparison of metal resistance of the bacterial isolates between sampling areas

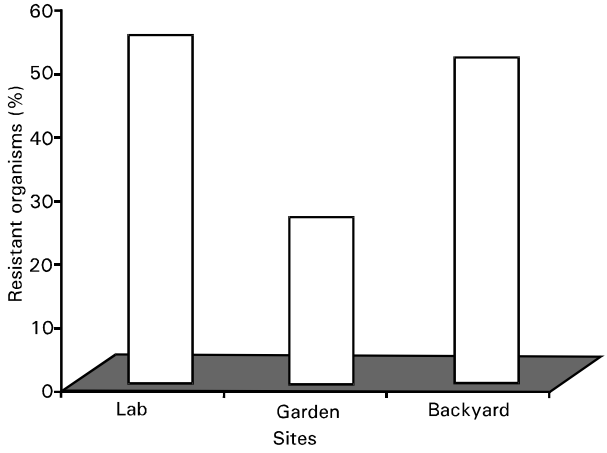

Fig. 4: Comparison of antibiotic resistance of the bacterial isolates between sampling areas

bacteria were pigmented whereas our results have shown that 80.7% were pigmented. The higher percentage of pigmented bacteria could be due to the high rate of pollution and bacteria normally produce pigments for their own protection from adverse effects of various agents. Pigmentation in environmental bacteria such as air and marine had been reported shown to protect them from the effects of solar/UV radiation. (Gregory, 1973 and Liu *et al.*, 1993).

In order to have an idea of the extent of pollutants of the sampling sites i.e, CMG Lab, CMG Garden and CMG Backyard, resistance was checked for heavy metal and antibiotics. Overall high degree of resistance against metals was observed in the sampling area of garden (Fig. 3). This may be due to the bus terminal near the sampling area. Buses at the terminal exhaust smoke all day which pollute the air. Garden isolates showed 16.66, 64.81, 66.66, 53.33 and 7.4% resistance to Ni^{+2}, Co^{+2}, Cu^{+2}, Cd^{+2} and Hg^{+3} respectively (Fig. 2). Highest resistance was observed against Ni^{+2} salt. Ni^{+2} is normally required in trace by bacteria and it is component of many enzymes but at 100μM concentration Ni^{+2} salt impair or suppress growth (Hausinger, 1987). 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} required in trace amounts as micro-nutrient. Antibiotic sensitivity was checked against antibiotics (Am, Km, Sm, Tc). However all the isolates showed a high degree of resistance against Am and Km (Fig. 1). Most of the isolates showed multiple antibiotic resistance. Highest degree of resistance was observed in lab isolates for tested antibiotics (Fig. 4), this may be due to the excessive use of antibiotics in the CMG lab. Lab isolates showed 84.3, 91.6 and 39.58% resistance to Km, Am and Sm respectively. Lab isolates showed highest resistance

against Am (Fig.1). There could be two possibilities firstly we have a good collection of environmental bacteria most of which are highly resistant to antibiotics and antibiotic resistance from these could have been transferred to airborne bacteria of the Lab. And secondly since antibiotics are used as a selection pressure their constant use might have resulted in the emergence of antibiotics resistance in airborne bacteria of the Lab. The differences observed in CMG Lab, CMG Garden and CMG Backyard (the sampling area) among antibiotic resistance and metal resistance (Table 2) was not significant. This may be due to small sample size or due to connection of three environments through windows and doors, which are frequently used.

It is concluded that air-borne bacteria at different locations of CMG in respect to metal and antibiotic resistances have shown variability. Higher metal resistance was observed among bacterial isolates of garden and backyard, whereas higher resistance to antibiotics was observed among the bacterial isolates of lab. The reason could be because backyard and garden are close to bus terminal of the University and for antibiotic resistance among lab isolates could be attributed to the constant use of antibiotics in the lab for research purposes. Since bacteria could be used for monitoring pollution these studies have provided an idea of environmental status of the three locations of the University.

References

Ahmed, N., F. Fasim, M. Arif and N. Jamil, 2000. Inducible Metal tolerance to heavy metals in air borne bacteria. Pak. J. Biol. Sci., 3: 2232-2237.

Conover, W.J., 1980. Kruskal-Wallis test (several independent samples) In: Practical non parametric statistics, second ed. John Wiley and Sons, Inc., pp: 229-231.

Gregory, P.H., 1973. Microbiology of the atmosphere. John Wiley and Sons, New York.

Hausinger, R.P., 1987. Nickle utilization by microorganisms. Microbiol. Rev., 51: 22-42.

Liu, Y.T., M.J. Sui, D.D. Ji, I.H. Wu, C.C. Cou and C.C. Chen, 1993. Protection of ultravoilet irradiation by melanin of mosquitocidal activity of *Bacillus turingensis var. israelensis*. J. Invert. Path., 62: 131-136.

Maniatis, T., E.F. Fritsch and J. Sambrook, 1982. Molecular cloning a laboratory manual. Cold Spring Harbour Laboratories Publications, pp: A.6

Marthi, B. and B. Lighart, 1990. Effects of betaine on enumeration of airborne bacteria. Appl. Environ. Microbiol., 56: 1286-1289.

Marthi, B., B.T. Shaffer, B. Lighart and L. Ganio, 1991. Resuscitation effects of catalase on airborne bacteria. Appl. Environ. Microbiol., 57: 2775-2776.

Mergeay, M., D. Nies, H.G. Schlegel, J. Greits, P. Charles and F. Van Gijsegem, 1985. Alcaligenes eutrophus CH34 is a facultative chemolithotroph with plasmid bound resistances to heavy metals. J. Bacteriol., 162: 328-334.

Nakara, H.,T. Ishikawa, Y. Sarai, I. Kondo and S. Mitsuhashi, 1977. Frequency of heavy metal resistance in bacteria from inpatients in Japan. Nature (London), 266: 165-167.

Raihan, S., F. Sarwar and N. Ahmed, 1995. Isolation, characterization and assessment of metal accumulation of bacterial isolates from industrial waste. In Biotechnology for Environment and Agriculture, Ahmed, N., M. Ishaq, O.Y. Khan, and F. Sarwar (eds), University Press, Karachi, pp: 143-154.

Russell, M.P., J.A. Goldsmith and I. Phillips, 1984. Some factors affecting the efficiency of settle plates. J. Hospital Infection, 5: 189-199.

Rheinheimer, B., 1974. Aquatic microbiology, John Wiley and Sons, London, pp: 80 -82.

Shaffer, B. T. and B. Lighart, 1997. Survey of culturable airborne bacteria at four diverse location in Oregon: urban, rural, forest and coastal. Micrb. Ecol. Nov., 34: 167-177.

Summers, A.O., G.A. Jacoby, M.N. Swartz, G.Mc. Hugh and L. Sutton, 1978. Metal cation and oxynion in plasmids of gram-negative bacteria. American Soc. Microbiol., 128-131.