



*Original Contribution*

## MULTIPLE ANTIBIOTIC RESISTANCE OF ENVIRONMENTAL BACTERIA ISOLATED FROM HEAVY METAL POLLUTED INDUSTRIAL REGION

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### ABSTRACT

Present study investigated the antibiotic resistance profile of bacterial strains isolated from soil, sediment and waters of Eloor-Edayar Industrial belt of Kerala, South India. A total of 165 bacterial strains were isolated and tested for their sensitivity to 11 common antibiotics (Ampicillin, Erythromycin, Gentamycin, Nalidixic acid, Penicillin, Vancomycin, Novobiocin, Amikacin, Kanamycin, Streptomycin and Ciprofloxacin). Results of the present study revealed that all of the isolates were resistant to at least one antibiotic tested. In soil isolates, resistance to penicillin (85.41%), novobiocin (66.67%) and nalidixic acid (60.41%) was noted most commonly and least resistance was observed against gentamicin (10.41%), amikacin (14.58%) and ciprofloxacin (18.75%). Isolates from sediments were highly resistant to penicillin (97.87%), ampicillin (78.72%) and nalidixic acid (44.68%) and low resistance was against ciprofloxacin (14.89%), amikacin (17.02%) and gentamicin (19.14%). Bacterial strains from water showed their high resistance against kanamycin (98.59%), penicillin (97.18%) and novobiocin (80.28%) and least resistance against gentamicin (32.39%), amikacin (33.80%) and ciprofloxacin (35.21%). A total of 31, 35 and 50 different resistant patterns were observed among the isolate from soil, sediment and water respectively. Most frequent resistant patterns were NaPNv (12.90%), PAm (20%) and AkVaNaPKGmNvSCfAm (22%) among the isolates of soil, sediment and water respectively. Many isolates had acquired multiple drug resistance (MDR). Exposition of multiple antibiotic resistance (MAR) index and resistance patterns of every isolates, which revealed high prevalence of MAR among the isolates encountered in the present work.

**Key words:** Water, soil, sediment, pollution, bacteria, antibiotic resistance

### INTRODUCTION

Antibiotic resistance will eventually occur because of evolutionary natural selection, but the misuse and overuse of antibiotics is dramatically escalating the process. In recent years, much attention has been given to the increase in antibiotic resistance. As more microbial species and strains become resistant, many diseases have become difficult to treat, a phenomenon frequently ascribed to both indiscriminate and inappropriate use of antibiotics in human

medicine. However, the use of antibiotics and antimicrobials in raising food animals has also contributed significantly to the pool of antibiotic resistant organisms globally and antibiotic resistant bacteria are now found in large numbers in virtually every ecosystem on earth.

Resistance to antibiotics is acquired by a change in the genetic makeup of a bacterium, which can occur by either a genetic mutation or by transfer of antibiotic resistance genes between bacteria in the environment. Products such as disinfectants, sterilants, and heavy metals used in industry and in house hold products are, along with antibiotics, creating a selective pressure in the environment that leads to the mutations in

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microorganisms that will allow them better to survive and multiply (1).

Concern over resistance was originally confined to acquisition of resistance by microorganisms which cause epidemic disease and was an issue only with respect to clinically isolated strains. However, in recent years, antibiotic resistant bacteria have been isolated from virtually every environment. Resistance genes can be spread far wider than once believed and a pool of resistance is developing in non-pathogenic organisms found in humans, animals, and the environment. These non-pathogenic organisms serve as a source from which pathogens can acquire genes conferring resistance, and in turn, they can become resistant by acquiring genes from pathogens discharged into the environment,

Antibiotic resistance studies in the bacterial community of the metal contaminated areas are very important because many researchers suggested that metal exposure indirectly selects for bacteria resistant to unrelated toxicants, particularly antibiotics (2). Multiple genes encoding for metal and antibiotic resistance are commonly found on the same plasmids or transposons, for example, transposon Tn21 conferring co-resistance to mercury, aminoglycosides, and sulfonamides (3). In some cases, single enzymes function as efflux pumps for multiple metals and antibiotics; this is defined as cross-resistance (4). In either case, direct selection for metal resistance could indirectly select for organisms or mobile genetic elements conferring antibiotic resistance. Accordingly, elevated frequencies of antibiotic resistance have been observed in the culturable fractions of microbial assemblages in metal-contaminated freshwater streams (5). For the present study we select metal contaminated Eloor-Edayar Industrial belt of Kerala near Cochin City for sample collection.

## MATERIALS AND METHODS

**Study area:** For the present study we select Eloor-Edayar industrial area - largest industrial belts of Kerala for the sample collection. Eloor otherwise known as Udyogmandal is the houses of FACT, TCC, IRE, HIL and many other small and big industries. Eloor is an island of 14.21 km<sup>2</sup> formed between two distributaries of river Periyar. Kerala pollution control board studied the heavy metal contamination in the

same area and reported that area was highly polluted with heavy metals.

**Collection of Samples:** Soil, sediment and water samples were collected from abandoned paddy fields, canals and river of the selected industrial area. Totally seven sampling sites were identified and from each sampling site soil, sediment and water samples were collected. Soil samples were collected at a depth of 15 to 20 cm from the surface after removing the top layer. For each of the sampling sites, sub-samples of soil were collected from different locations, pooled together and homogenized so as to obtain representative sample. Samples were collected using a spade that is thoroughly cleaned and disinfected between sampling so as to prevent cross-contamination. Sediment samples were collected using Grab sampler and transferred to sterilized plastic bags. Water samples were collected using sterilized plastic bottles. Soil, sediment and water samples were transferred to an ice box and transported to the laboratory.

**Isolation of bacteria:** Isolation and enumeration of bacteria were carried by standard serial dilution plate technique. For soil and sediment, 10 g of samples were transferred into 90 ml sterile distilled water and agitated vigorously and for water samples 1ml of samples were transferred into 99ml sterile distilled water. Serially diluted samples were sow in Nutrient Agar and incubated at 37°C for 24- 48 hours. Bacterial colonies from Nutrient agar were isolated, purified and maintained as a pure culture for further study.

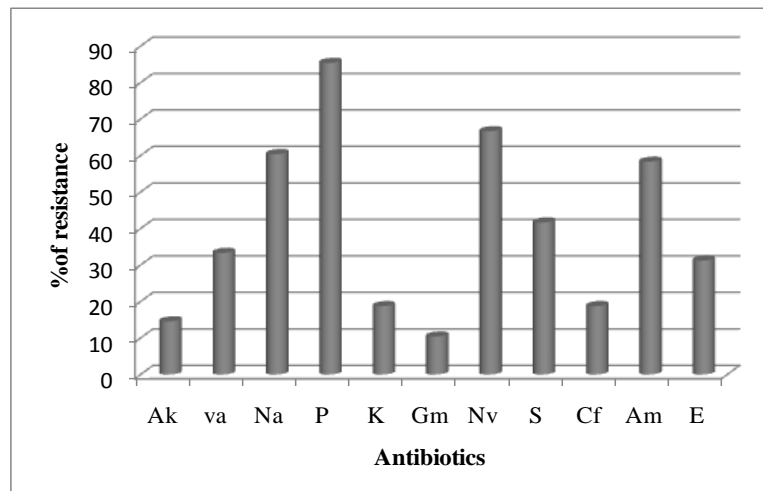
**Antibiotic resistance analysis:** Antibiotic sensitivity testing of the bacterial isolates was carried out by disk diffusion method as per Bauer *et al.* (6). Briefly, the isolates were grown in nutrient broth for 16-18 hours. The grown cultures were then swabbed evenly onto the surface of Mueller-Hinton agar using sterile cotton swabs. After 15 minutes of pre-diffusion time, antibiotic impregnated paper discs were placed on the surface of the agar using a sterile forceps and the plates were incubated at 35°C for 24 h. After incubation the diameter of zone of inhibition was measured to the nearest millimeter. Antibiotics and their doses tested were as follows: Ampicillin (10 mcg/disc), Erythromycin (15mcg/disc), Gentamycin (10mcg/disc), Nalidixic acid (30mcg/disc),

Penicillin (10units/disc), Vancomycin (30mcg/disc), Novobiocin (30mcg/disc), Amikacin (30mcg/disc), Tetracycline (30mcg/disc), Streptomycin (10mcg/disc), Ciprofloxacin (5mcg/disc). Antibiotic resistance was determined by comparing bacterial isolate inhibition zone diameters to established values.

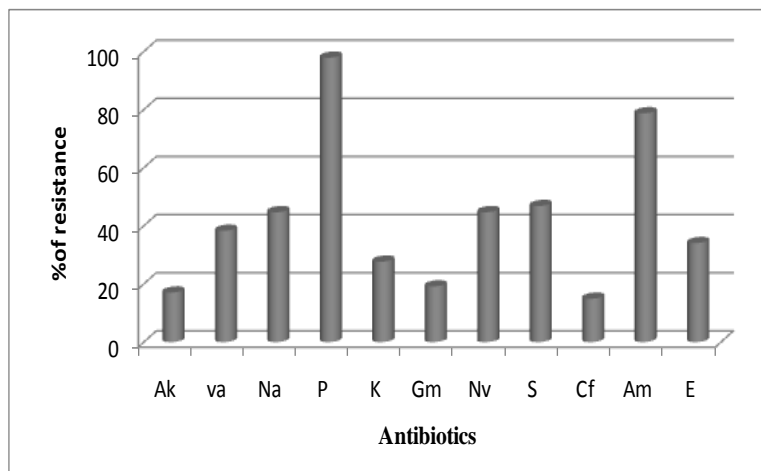
**Multiple Antibiotic Resistance (MAR) indexing of the isolates:** The MAR index when applied to a single isolate is defined as a/b where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the total number of antibiotics to which the isolate was exposed.

**RESULTS AND DISCUSSION**

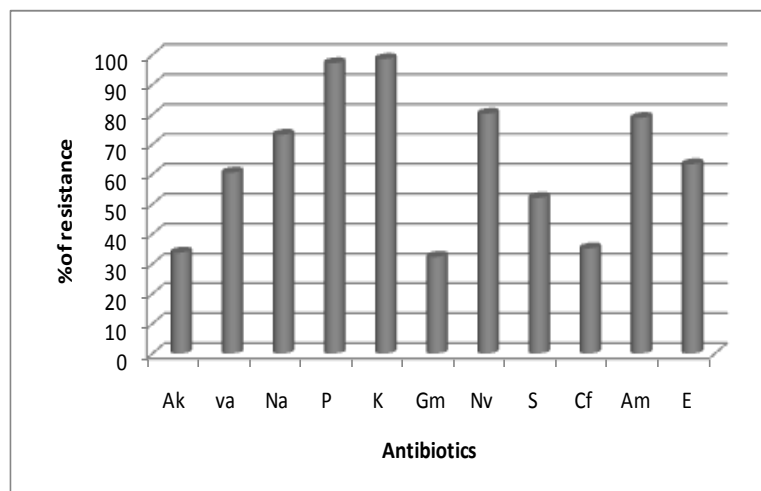
A total of 165 bacterial strains were isolated from the sediment, soil and water and tested for their sensitivity to 11 antibiotics such as ampicillin, erythromycin, gentamicin, nalidixic acid, penicillin, amikacin, novobiocin, vancomycin, streptomycin, kanamycin and ciprofloxacin that are widely used in treatment of diseases in human as well as in animal production systems. All the isolates were resistant to at least one antibiotic. Isolates from soil were resistant to penicillin (85.41%), novobiocin (66.67%) and nalidixic acid (60.41%) most frequently and least resistance was observed against gentamicin (10.41%), amikacin (14.58%) and ciprofloxacin (18.75%) (**Figure 1**). In sediment isolates, resistance to penicillin (97.87%), ampicillin (78.72%) and nalidixic acid was noted commonly (44.68%) and low resistance was against ciprofloxacin (14.89%), amikacin (17.02%) and gentamicin (19.14%) (**Figure 2**). Bacterial strains from water showed their high resistance against kanamycin (98.59%), penicillin (97.18%) and novobiocin (80.28%) and least resistance against gentamicin (32.39%), amikacin (33.80%) and ciprofloxacin (35.21%) (**Figure 3**). A total of 31, 35 and 50 different resistant patterns were observed among the isolate from soil, sediment and water respectively. Most frequent resistant patterns were NaPNv (12.90%), PAm (20%) and AkVaNaPKGmNvSCfAm (22%) among the isolates of soil, sediment and water isolates respectively.



**Figure 1.** Percentage of antibiotic resistance of bacterial isolates from Soil



**Figure 2.** Percentage of antibiotic resistance of bacterial isolates from Sediment samples



**Figure 3.** Percentage of antibiotic resistance of bacterial isolates from Water samples

Our finding of elevated microbial tolerance to antibiotics in metal-contaminated industrial area by culture dependent methods agrees with culture-based determinations of resistance among isolates obtained from metal-contaminated coastal areas (7, 8) and freshwater streams (5). Results of the present study in general consistent with those of other studies in which varying frequencies of antibiotic resistance were detected with bacteria in natural environments (9, 10, 11). Many isolates had acquired multiple drug resistance (MDR). Elucidation of multiple antibiotic resistance (MAR) index and resistance patterns of each isolates, which revealed high prevalence of MAR among the isolates encountered in the present study. D'Costa, *et al.* (9) reported varying frequencies of MAR in soil microbes.

Different antibiotic resistance patterns of bacterial isolates may be due to the difference in the selection pressure for the emergence of resistant mutants. There has been considerable concern regarding environments containing antibiotics due to the possibility of antibiotic-resistant strains becoming dominant in the bacterial communities in such ecosystems (12, 13, 14, 15). The frequencies of resistance and MAR at the soil could, in part, have been influenced by the use of antibiotics in animal feeds, because it is believed to increase resistance among bacteria in surface soils and other environments (16, 11). Although resistant organisms can be found naturally in the environment, most resistance is associated with man-made impacts of some type, either agricultural or direct human impact.

## CONCLUSIONS

In conclusion most of the bacterial strains studied in the present work had acquired multiple drug resistance (MDR). Elucidation of multiple antibiotic resistance (MAR) index and resistance patterns of each isolates, which revealed high prevalence of MAR among the bacterial isolates encountered in the present study. This may be due to the metal induced indirect selection for antibiotic resistance.

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