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ANTIBIOTIC RESISTANCE OF HETEROTROPHIC BACTERIA ISOLATED FROM WATER AND BLACK CLAM IN COCHIN BACKWATER

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ABSTRACT

Bacterial strains isolated from water and the black clam *Villorita cyprinoides* var. *cochinensis* (Hanley) were tested for resistance to ten antibiotics namely ampicillin, bacitracin, chloramphenicol, gentamycin, neomycin, oxytetracycline, penicillin-G, polymyxin-B, streptomycin and sulphadiazine. Maximum number of strains (95.4%) showed resistance to penicillin. The resistance pattern was found to be Penicillin>Sulphadiazine>Ampicillin>Bacitracin>Streptomycin>Neomycin>Polymyxin-B>Oxytetracycline>Chloramphenicol>Gentamycin. The least resistance was offered to gentamycin by all the genera except *Flavobacterium*/*Cytophaga* group, which showed least resistance to Polymyxin-B. Strains of *Vibrio* spp. and *Aeromonas* spp. exhibited maximum resistance to sulphadiazine, whereas rest of the strains showed maximum resistance to penicillin.

INTRODUCTION

A STUDY of drug resistant bacteria in Chesapeake Bay and the Atlantic Ocean has revealed that drug-resistant bacteria do exist in the marine environment and that plasmid-mediated drug-resistance does occur in marine bacteria (Colwell and Sizemore, 1974). With the exception of the large number of studies that have dealt with the antibiotic resistance of faecal coliform organisms isolated from nature, little is known about the antibiotic resistance pattern of gram-negative bacteria that occur in the environment (Kelch and Lee, 1978). The ability of various gram-negative bacteria to produce disease is well known (McCabe *et al.*, 1972) and the ability of resistance factor containing (R_+) bacteria to transfer drug resistance is also reported (Richmond, 1972). It is also proved that the R factors harboured by nonpathogenic intestinal bacteria such as *E. coli* can be transferred to drug-susceptible pathogens such as *Salmonella* or *Shigella* (Kasuya, 1964; Watanabe, 1971). In coastal regions and estuaries, the influx of domestic sewage provides a rich source of plasmid carrying bacteria (Colwell and Sizemore, 1974). Filter feeding shellfish tend to concentrate bacteria from overlying waters (Cooke, 1976). Goyal and Adams (1984) also reported that resistant bacteria may be concentrated by filter feeding shellfish, which inhabit waters overlying the dumpsites. Consumption of such shellfish by men may result in colonization of their gut by these bacteria. Although not pathogenic themselves, these bacteria may transfer their resistance to already colonized bacteria or other human pathogens. In India, studies on drug resistant bacteria are pertaining to certain groups of pathogens and indicators, and no information is available on the occurrence of drug resistant heterotrophic bacteria in the coastal environment. Hence it was planned to investigate the presence of drug resistant bacteria in Cochin backwater, to understand the pollution caused by drugs.

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Materials and Methods

A total of 505 strains of different genera were isolated from samples of water and of the black clam *Villorita cyprinoides* var. *cochinensis* (Hanley). The water and animal samples were collected from the Cochin backwaters near Kumbalam Island (Fig. 1). The isolated bacterial strains were identified upto the generic

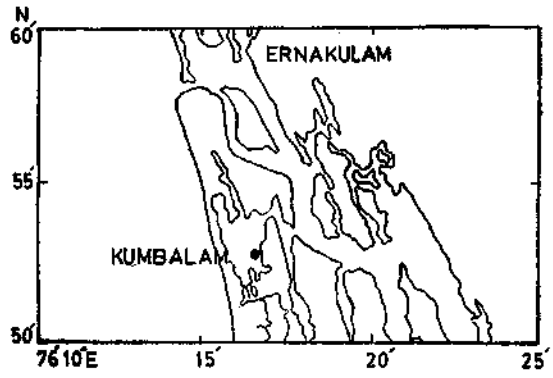

Fig 1. Position of the sampling station.

level (Shewan *et al.*, 1960; Buchanan and Gibbons, 1974). The strains were tested for antibiotic resistance, using agar diffusion technique. The antibiotics used and their concentrations per disc were ampicillin, 10 mcg; bacitracin, 10 units; chloramphenicol, 30 mcg; gentamycin, 10 mcg; neomycin, 30 mcg; oxytetracycline, 30 mcg; penicillin-G, 10 units; polymyxin-B, 300 units; streptomycin, 10 mcg; and sulphadiazine, 300 mcg (HI-MEDIA). Cultures were enriched in nutrient broth containing 1.5% NaCl at 30°C for 24 hrs. The enriched cultures were seeded over nutrient agar using sterilized cotton swabs. The discs of antibiotics were carefully placed immediately after inoculation on the agar medium using flammed forceps. They were kept at a minimum distance of 15 mm from the edge and sufficiently separated from each other to avoid overlapping of the inhibition zone. The discs were lightly pressed with the forceps to make complete contact with the surface of the medium. After 30 min., the plates were incubated at 30°C for 16-18 hrs. After incubation period, the zone of inhibition was measured and the isolates were classified into resistant, intermediate and sensitive.

Results and Discussion

Results of the resistance towards antibiotics exhibited by the bacterial strains are given in Fig. 2 and Table 1. For convenience, the strains showed intermediate reaction to various antibiotics were counted as resistant ones and discussed. Although the intermediate ones were considered along with the sensitive group by Trudel *et al.* (1984), it is proper to count them as resistant (Joseph 1974; Niemi *et al.*, 1983) since they are also equally important as resistant bacteria.

Of all the 505 strains tested, 95.4% were resistant to penicillin. The resistance pattern was as follows: Penicillin-G > Sulphadiazine > Ampicillin > Bacitracin > Streptomycin > Neomycin > Polymyxin-B > Oxytetracycline > Chloramphenicol > Gentamycin. Colwell and Sizemore (1974) reported that resistance to drugs such as penicillin is due to the acquisition of a penicillinase plasmid. The result is comparable with the findings of Pradeep and Lakshmanaperumalsamy (1985) on antibiotics resistance of *Vibrio parahaemolyticus* strains. They have reported 100% of their isolates to be resistant to penicillin. Also, the least percentage was resistant to gentamycin. Trudel *et al.* (1984) has also reported the penicillin resistant character by 100% of isolates tested. In our study, the high penicillin resistance could be attributed to the fact that penicillin is not commonly used against gram negative forms (Cruickshank *et al.*,

TABLE 1. *Percentage resistance of genera to different antibiotics*

Genus	G	P	C	O	N	S	Sz	A	B	PB
Pseudomonas ..	18.3	99.2	57.3	55.0	69.5	80.2	91.6	84.7	79.4	54.2
Vibrio ..	23.4	90.4	36.2	53.2	73.4	76.6	93.6	78.7	85.1	60.6
Aeromonas ..	31.3	95.5	53.7	53.7	74.6	79.1	97.0	83.6	86.6	56.7
Enterobacteriaceae ..	19.2	87.7	30.1	28.8	65.8	69.9	83.6	56.2	53.4	56.2
Acinetobacter ..	0.0	100.0	6.7	26.7	6.7	20.0	86.7	26.7	33.3	26.7
Alcaligenes ..	21.7	100.0	52.2	47.8	73.9	73.9	100.0	91.3	69.6	52.2
Flavobacterium/ *Cytophaga* group ..	88.9	100.0	88.9	88.9	88.9	100.0	100.0	100.0	88.9	44.4
Moraxella ..	11.1	94.4	77.8	94.4	83.3	61.1	83.3	83.3	55.6	72.2
Bacillus ..	15.0	100.0	50.0	25.0	55.0	50.0	100.0	95.0	75.0	80.0
Coryneforms ..	24.1	86.2	44.8	48.3	69.0	72.4	79.3	72.4	75.9	44.8
Micrococcus ..	11.5	100.0	26.9	34.6	34.6	88.5	100.0	88.5	76.9	26.9

G : Gentamycin P : Penicillin-G C : Chloramphenicol
O : Oxytetracycline N : Neomycin S : Streptomycin
Sz : Sulphadiazine A : Ampicillin B : Bacitracin
PB : Polmyxin-B

1976). Since more than 85% of our isolates were gram-negative, they might not have responded to penicillin and this might have been the reason for high (95.4%) occurrence of penicillin resistant bacteria.

Generawise resistance pattern showed some common characteristics. The least percentage was resistant to gentamycin. All the genera except *Flavobacterium-Cytophaga* group were falling in the above pattern. *Flavobacterium-Cytophaga* group have shown least resistance to polymyxin-B. Strains of *Vibrio* spp. and *Aeromonas* spp. exhibited maximum resistance to sulphadiazine whereas rest of the strains belonging to other genera showed maximum resitance to penicillin. However, *Vibrio* spp. and *Aeromonas* spp. recorded resistance to penicillin next to sulphadiazine. The result clearly points out that, in general, gram-positive and gram-negative bacterial strains except those belonging to *Flavobacterium-Cytophaga* group encountered in the investigation showed a similar pattern of resistance to various antibiotics. The antibiotic resistance pattern of gram-negative bacteria of different

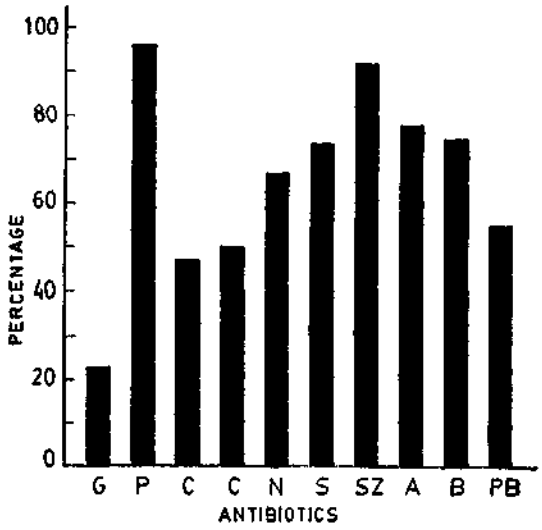

Fig. 2. Resistance of bacterial isolates to various antibiotics. (G : Gentamycin ; P : Penicillin-G ; C : Chloramphenicol ; O : Oxytetracycline ; N : Neomycin ; S : Streptomycin ; Sz : Sulphadiazine ; A : Ampicillin ; B : Bacitracin ; PB : Polymyxin-B).

genera tested by Kelch and Lee (1978) followed the same pattern, indicating that the bacteria which share a common environment also share a common mode for developing antibiotic resistance.

The extent of resistance to number of antibiotics by the bacterial strains showed that maximum number (21.8%) of strains were resistant to three antibiotics in different combinations, followed by combinations of four antibiotics (20.6%) (Fig. 3). Minimum (0.8%) was recorded by combinations of eight and nine antibiotics. 8.7% of the strains exhibited resistance patterns of PSSZAB which was found to be maximum for all combinations. 5.3% of the strains were found to be resistant to none of the antibiotics tested.

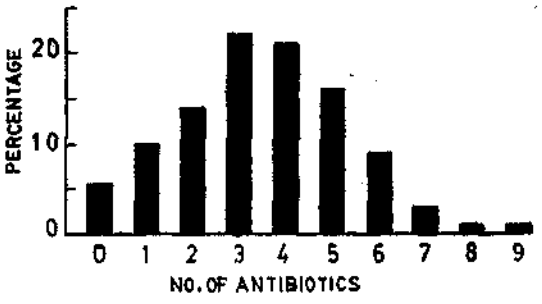

Fig. 3. Percentage occurrence of multiple resistance in bacterial isolates.

The resistance to antibiotics when acquired once by bacteria associated with organisms at higher trophic levels were seldom lost compared with those bacteria of water and sediment and organisms at lower trophic levels, where they have to endure more environmental stresses (Pradeep and Lakshmanaperumalsamy 1985). Colwell and Sizemore (1974) has reported that the frequencies in antibiotic resistance among bacteria were seen to be dependent on the amount and kinds of antibiotics used in the area.

As the black clam is a major food item of the inhabitants of coastal areas of Cochin, there is a possibility of ingesting antibiotic resistant bacteria which may pose health hazard to animals and man. Also this water body is extensively used by man for various activities such as fishing, recreation, transport etc. Precautionary measures have to be taken immediately to check the inflow of domestic sewage containing this contaminants to avoid any untoward situations.

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