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Evaluation Of Antibiotics Tolerance In Isolated Pure Bacterial Cultures From Various Places Form Arabian Costal Around Karwar District, Karnataka, India.

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ABSTRACT

The coastal ecosystem is a rich source of microorganisms; Karwar of India facing the Arabian Sea is one among such biological hot spots due to the interface of sea and species rich Western Ghats. Coastline beaches are a rich source of microorganisms. Among such microorganisms, bacteria are of particular interest, since they play a decisive role in the cycle of matter in water. Identification and characterization of isolated pure bacterial cultures were performed by biochemical tests and morphological characters. The maximum tolerable concentration for antibiotics were carried out on the 42 important marine bacteria isolated from Binaga beach, Bilikari beach and Majali beach near Karwar, Karnataka. In a summary, the present study explores the extensive use of antibiotics in medicine, agricultural and aqua cultures have increases the occurrence of antibiotic resistant bacteria in future.

Keywords: Arabian costal Karwar, Bacterial biodiversity, Characterization, Antibiotic resistance, Antibiotics

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INTRODUCTION

Growing industrialization was useful to human beings; by the meantime, it also creates lots of problems in the environments. For examples, antibiotics, pharmaceutical and personal care products etc., were synthesized in the large amount and are used in healthcare to cure illness and finally undigested part of compounds with their byproducts were excrete and transferred through sewage systems into wastewater treatment plants (WWTPs). On the other hands, in WWTPs, these micropollutants were digested by abiotic and biotic methods. However, some of them were not completely removed. This results in the discharge of such pollutants (ranging from micro mole to nano mole) into aquatic environment and surrounding living systems. There are reports on micropollutants have shown their toxic effect on aquatic organisms [1, 2]. On other hand, there are reports on the degradation of micropollutants like triclosan, triclocarban, sulfamethoxazole and sulfadiazine in bacteria, which were isolated from wastewater, activated sludge and sediment [3-7]. The role of microbes both on land and water have ameliorating effects of naturally occurring and manmade disturbed environments. Because microorganisms are important source of knowledge about strategies and limits of life. In this paper, we isolate and identify the bacteria. Isolated bacteria is used to evaluate the antibiotic sensitive and resistance. Know day study of antibiotic sensitive and resistance is very important for the treatment and control of diseases as well as in maintaining the health of near shore ecosystems.

MATERIALS AND METHODS

Source

The water and sediment samples collected from Binaga beach, Bilikari beach and Majali beach near Karwar, Karnataka. About 10 gm of sediment and 10 ml of water were collected by using clean, dry and sterile brown colored glass bottles and the collected samples were immediately transported to the laboratory where they were kept in freezer at 4 $^{\circ}$C. The pure bacterial cultures were isolated by spread plate method as described previously [10].

Isolation and identification of pure organisms

The collected samples were transferred separately into a beaker containing sterile double distilled water. The final volume of each sample will be makeup into 100 ml by adding sterile double distilled water. From this, 100 µl of each dilution (samples) were inoculated in nutrient agar (NA) plates. Finally, the inoculated NA plates were incubated at 37 $^{\circ}$C for 48 h in incubator. The colonies with different colored were appeared on NA plates are chosen and transferred to NA plates for further purification. This process was carried out for several times to confirm the purity of each culture. The pure bacteria were preserved on nutrient agar slant. The isolated pure microorganisms morphological and Biochemical features were studied according to standard protocols.

Maximum tolerable concentration for antibiotics

To evaluate each organisms' maximum tolerable concentrations for antibiotics. The isolated pure bacteria were tested for their sensitivity and resistivity on antibiotics like tetracycline (TET), streptomycin (STP), norofloxin (NOR), ampicillin (AMP), ciproflxin (CIP), gentamicin (GEN), chloramphenciol (CHL) and pencillin (PEN) on nutrient agar plate by Well-diffusion method [11]. The concentration ranges from 50 µg /ml to 900 µg /ml.

RESULTS AND DISCUSSION

The microbes were isolated from water or/and sediment samples collected from zonal regions of coastal area of Arabian sea around Karwar such as from Binaga beach, Bilikari beach and Majali beach. The morphological and biochemical features of the isolated microbes were studied by implementing the standard methods [12-14]. The isolated bacteria were some of them gram-positive and gram-negative. According to Bergey's manual of determinative bacteriology, 9th edition (Holt,1993), the isolated microbes were tentatively identified as *Bacillus circulans, Bacillus alveli, Bacillus marinus, Bacillus meagaterium, Bacillus substilis, Bacillus cercus, Bacillus fastidiosus, Bacillus psychrophilus, Bacillus neizhouensis, Bacillus thuringiensis, Bacillus*

mycodes, Bacillus brevis, Bacillus lichenformis, Salimicrobium halophilum, Bacillus sp.*, Bacillus amyloliquefaciens, Enterococcus durans*, *Lyinibacillus sphaericus*, *Micrococcus* sp.*, Micrococcus lylae, Micrococcus lutes, Micrococcus caseolyticus*, *Micrococcus mucilaginosus*, *Pseudomons alcaligenes*, *Pseudomonas putida, Pseudomonas panacis, Staphyllococcus* sp., *Staphyllococcus aureus, Staphylococcus arlettae, Staphylococcus epidermidis, Streptococcus faecalis, Enterococcus hirae, Vibrio damsel, Vibrio fischeri, Vibrio proteolyticus, Vibrio alginolyticus, Vibrio metschnikovi, Tatumella punctata, Photobacterium angustum, Photobacterium* sp., *Shewanella putrafaciens* and *Arthrobacteria.* This investigation indicates, marine environment has predominant of *Bacillus* sp. compare to other species [15].

The response of organisms to different antibiotics was tested on nutrient agar medium. Inhibition of growth was depicted by a clear zone formation around the well indicated sensitive reaction, otherwise the organism was resistant to the antibiotics. The diameter of inhibition zone was measured with an antibiotic zone scale. *Arthrobacteria, Bacillus amyloliquefaciens, Bacillus psychrophilus, Pseudomonas alcaligenes, Shewanella putrafaciens,* and *Staphylococcus epidermis* are sensitive to the lower concentration of antibiotics like TET, STP, NOR, AMP, CIP, GEN, CHL and PEN as shown in Figure 1-3. On the hand, other isolated pure cultures were shown resistivity against higher concentrations of these individual antibiotics (Figure 1-3).

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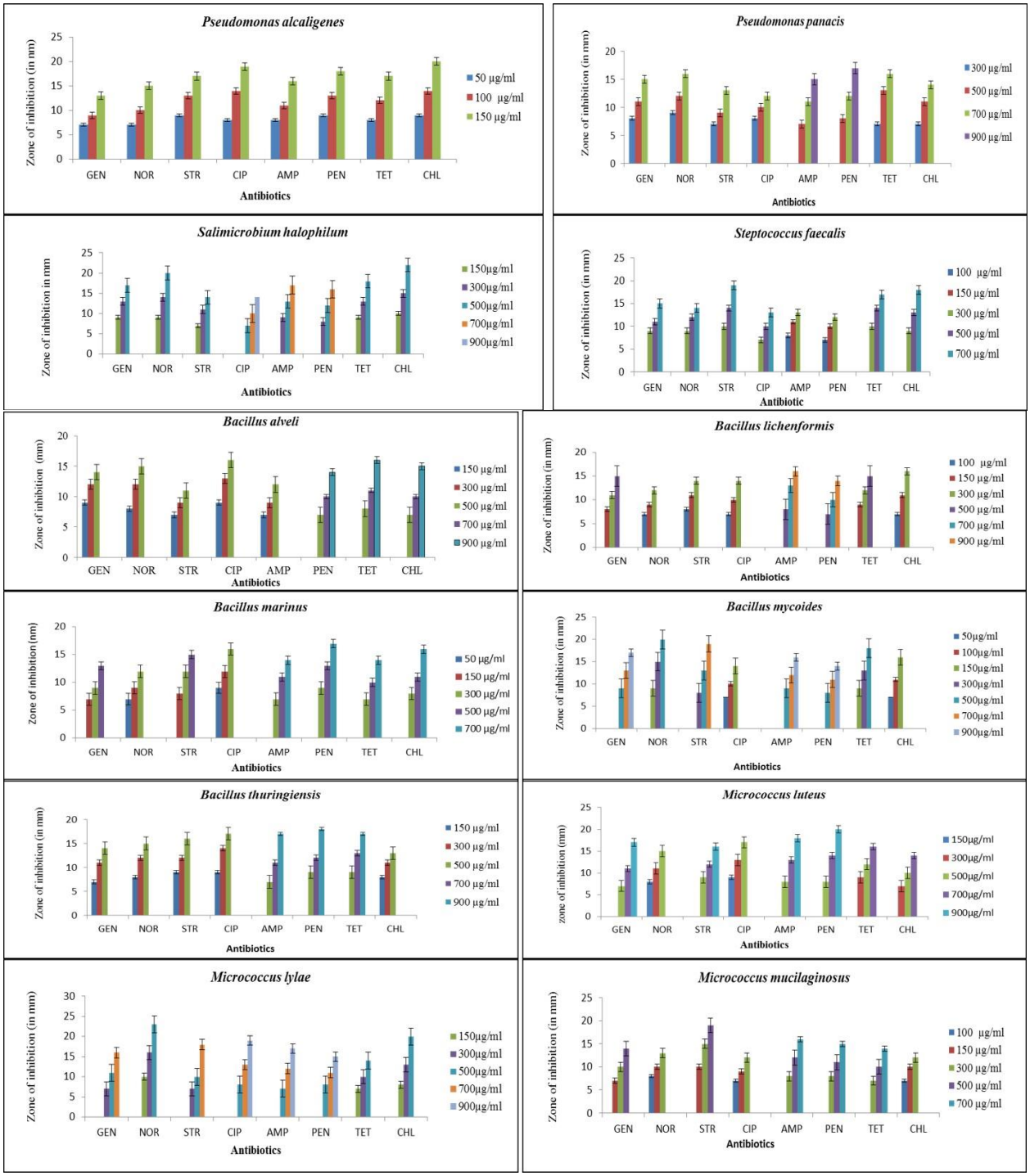

Fig 1: Susceptibility test of various pure isolates (Binaga beach) against different antibiotics.

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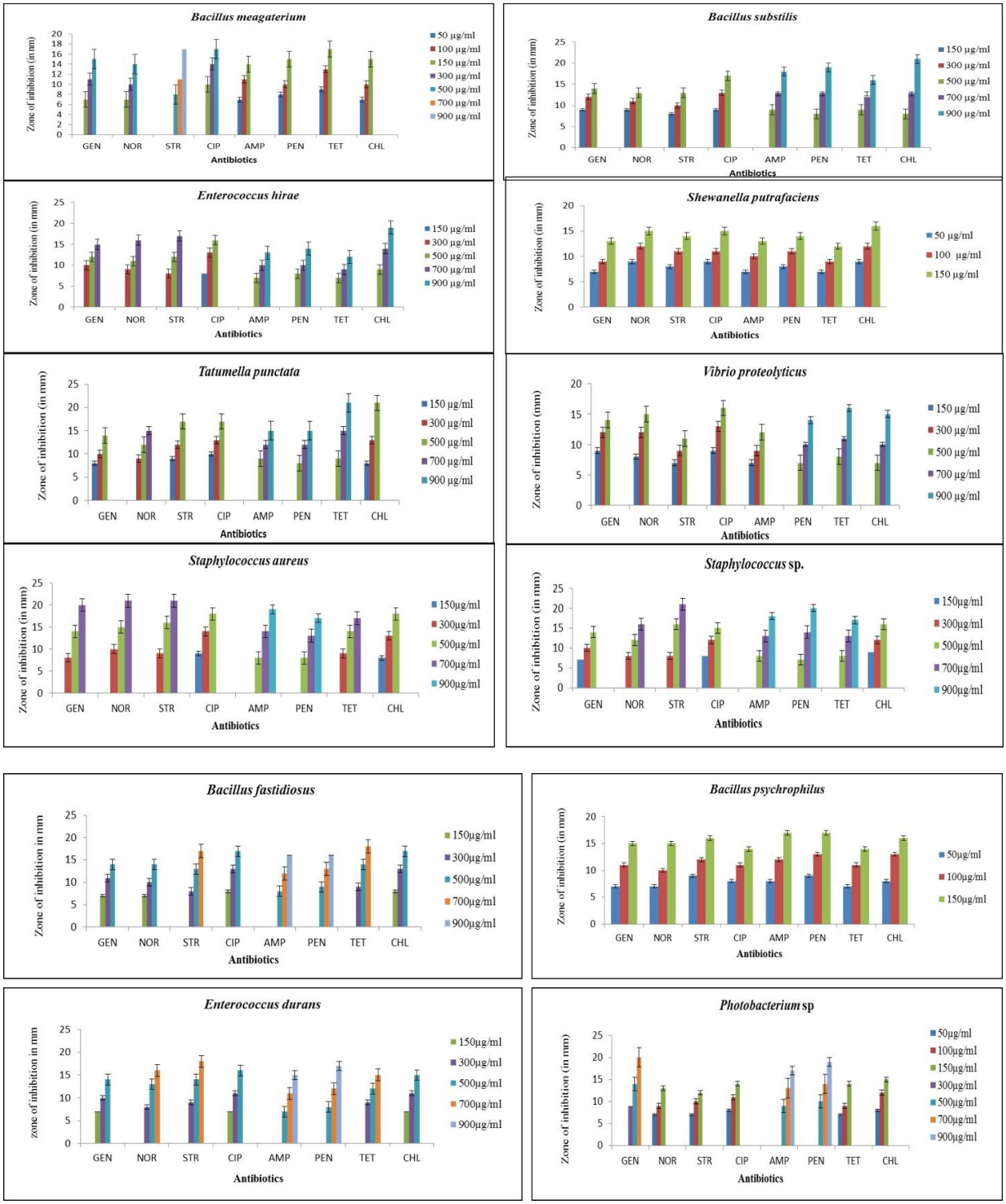

Fig 2: Susceptibility test of various pure isolates (Bilikari beach) against different antibiotics.

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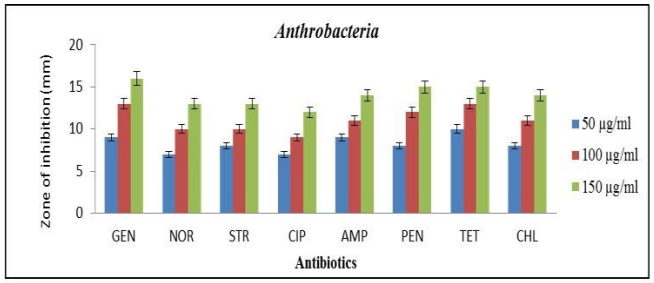

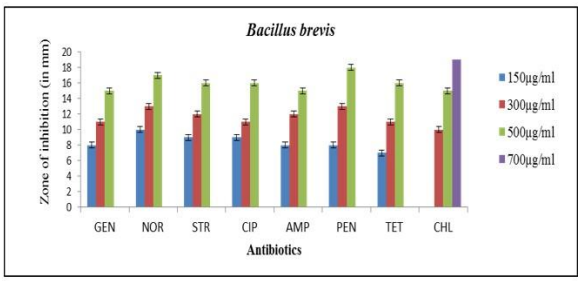

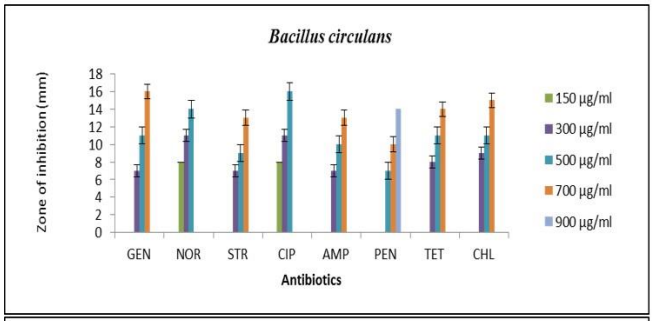

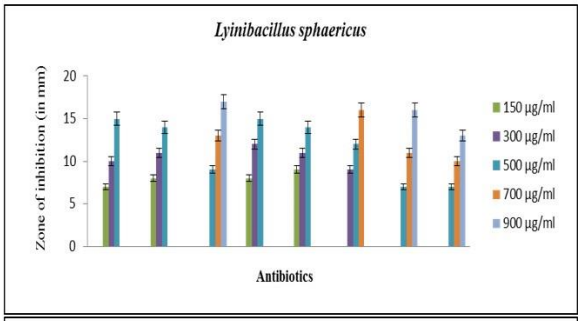

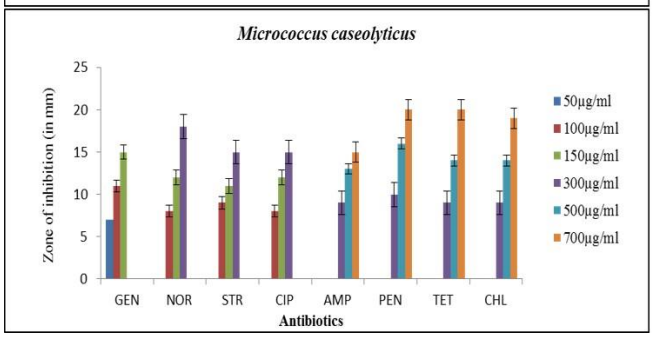

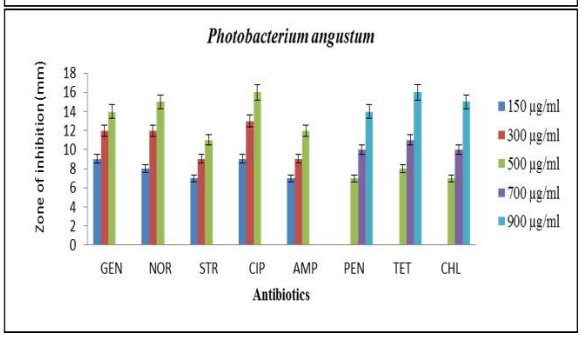

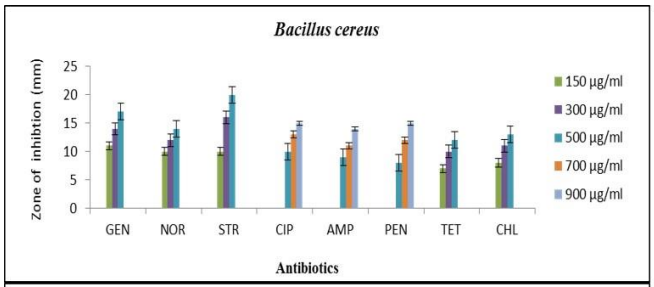

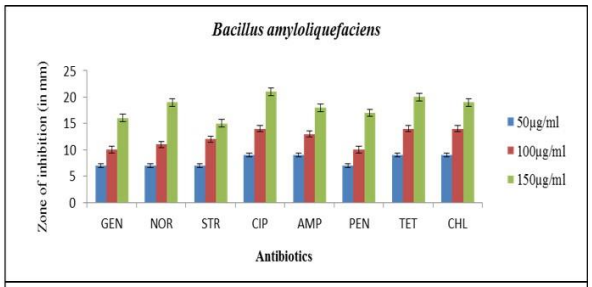

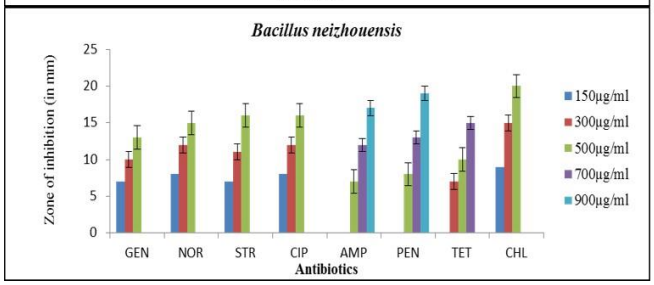

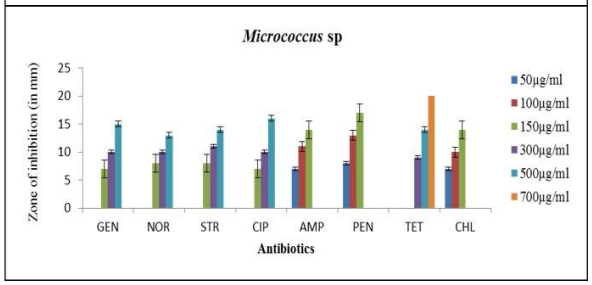

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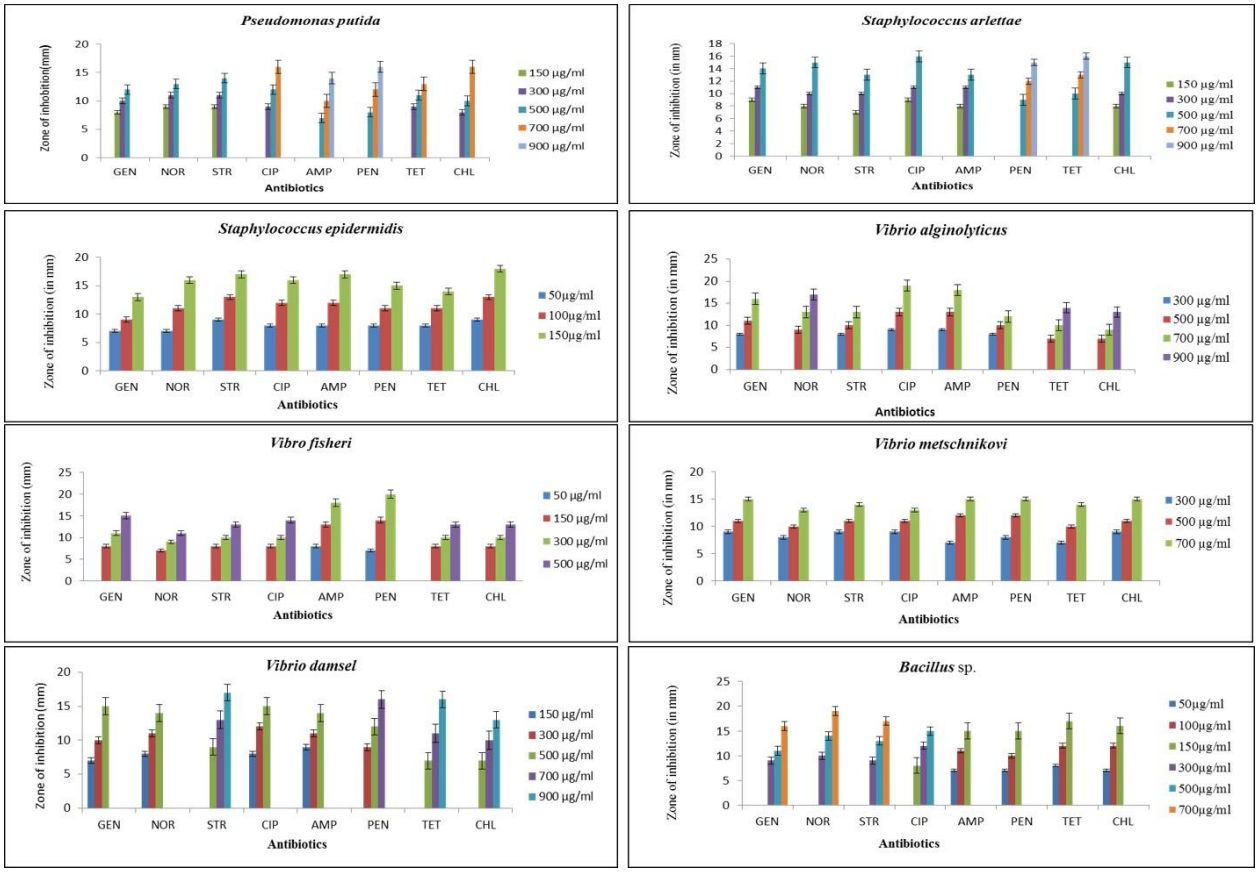

Fig 3: Susceptibility test of various pure isolates (Majali beach) against different antibiotics.

Extensive use and misuse of antibiotics in medication, agricultural and aquaculture have increased the occurrence of antibiotic resistance bacteria [16] in the natural environment. Dissimilarities in resistivity (and/or susceptibility) among the pathogenic organisms against antibiotics has become major problems in pharmaceutical active drug selection. Hence, antibiotic resistance bacteria in future complicating disease prevention and treatment [17]. Various researcher reported that high incidences of antibiotic resistant bacteria in marine environment [18-20]. The detail study was required to determine the distribution of antibiotic resistant bacteria in marine and estuarine environment for prevention and treatment for diseases.

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