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Twenty five numbers of crabs (*P. sanguinolentus*) were collected from Parangipettai fish market. *Paenibacillus* sp. were isolated from the gut of crabs. Then the isolated colonies were characterized for phenotypic and biochemical

K d : *Pae bac* sp; Recombination; *P.* *a* *e* ; Plasmid-borne drug resistant bacteria; Resistant bacteria

I d c

Antibiotic resistance is now a linked global problem. Dispersion of successful clones of multidrug resistant (MDR) bacteria is common, o en via the movement of people [1]. Antimicrobial resistance increases the morbidity, mortality and costs of treating infectious diseases. e threat from resistance (particularly multiple resistances in bacterial strains that have disseminated widely) has never been so great. e key factors driving this threat are increased antibiotic usage (in both human and animal medicine), greater movement of people and increased industrialization. Ine ective of the antibiotics against the bacterial infection is known as resistance [2]. Bacterial resistance is increasingly seen as a public health threat since few new antibiotics options are being introduced. e Infectious Diseases Society of America (IDSA) education campaign of "Bad Bugs, No Drugs" was begun to raise public awareness of this problem. ere are many factors that could be responsible for the increase in antibiotics resistance in developing countries [3]. erefore, the objective of the present study was undertaken to assess the multiple antibiotic resistance of bacteria isolated from the crab *P. a* *e* and focuses on the activity of bacteria to transform R-plasmid to *E.c.* in natural conditions.

Ma a a d M d

C c a d c a

e crab samples were collected from sh market, Parangipettai (Lat.11˚29'N; Long.79˚46' E), Tamil Nadu. Twenty ve crab samples were collected with meticulous care aseptically and transported in a sterile poly bag in ice to laboratory.

I a c b a

ey were washed several times with sterile sea water to prevent contamination from shell surface and mantle uid and subsequently the gut of the crabs were aseptically removed. e tissues adhering to the gut were carefully removed using a sterile forceps. e gut alone was homogenized with 9 ml of 50% sterile sea water. Serial dilutions were made from homogenate and from that 0.1 ml were spread into petriplates containing Zobell's marine agar for enumeration of *Pae bac* sp.

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as control. e cherry red ring formation indicates positive reaction and recorded as (+) and the absence was recorded as (-).

To the MR-VP broth, the isolates were inoculated and incubated at room temperature for 24-48 h and a er incubation few drops of methyl red indicator was added. MR-VP broth without the isolate inoculation was used as control. e colour change of broth culture to red colour was indicated as MR positive (+) and the absence indicated as negative reaction (-).

4 hours fresh culture of isolates. By making use of template drawn commercial antibiotics disc were dispensed on the solidi ed Muller Hinton agar. is was incubated at 37˚C for 24 hours in an incubator and was observed for the development of clearance/inhibition zones around the antibiotic disc.

e cells were grown overnight in Luria Bertani broth containing sodium chloride (LBS, NaCl, 2% w/v) and incubated at 37˚C in a shaker incubator (120 rpm) for 16–18 h. 1.5ml of culture were used for plasmid isolation by using Plasmid Isolation minispin kit (Chromous Biotech, Bangalore).

A bacterial colony was picked and streaked onto LB agar plate containing antibiotic and incubated for 24 hours. A er incubation the culture (*Pae , bac,,* sp.) were transferred into 5 ml of LB broth and incubated overnight at 37˚C in a shaker at 200 rpm. From that 1.5 ml of the culture was transferred into an eppendorf tube and centrifuged at 10,000 rpm for 1 min at 4˚C.

e supernatant was removed and pellet was collected. e pellet was then suspended in 250 µl of DNA suspension bu er (in cold condition). e bacterial cells were completely suspended by vortexing until no clumps remain. 250 µl of DNA extraction bu er was added and gently mixed by inverting 3-4 times. Immediately 350 µl of Plasmid Binder was added and mixed immediately by gently inverting the vials 3-4 times. e vials were then centrifuged at 13,000 rpm for 10 min at room temperature. e supernatant containing plasmids were decanted into an empty spin column along with the collection tube (600 µl at a time). e column was centrifuged at 13,000 rpm for 1 min at room temperature. e contents of collection to tubes were discarded and the spin column was placed back in the same collection tube. 500 µl of clean-up bu er was added to the column and spinned at 13,000 rpm for 1 min at room temperature. e contents of collection to tubes were discarded and the spin column was placed back in the same collection tube. 500 µl of 1X wash bu er was added to the column and spinned at 13,000 for 1 min at room temperature. e contents of collection to tubes were discarded and the spin column was placed back in the same collection tube. e empty column along with collection tube was centrifuged at 13,000 rpm for 3 min at room temperature. e spin column was then replaced in a 1.5 ml fresh eppendorf tube. 50 µl of elution bu er was added to the centre of the membrane of spin column. e vial along with spin column was kept at room temperature for 2 min and then centrifuged at 15,000 rpm for 1 min at room temperature. e spin column is then removed from the vial and the isolated plasmid was collected in the eppendorf tube. e isolated plasmid was then subjected to electrophoresis.

Electrophoresis was performed using 0.8% agarose gel system (Bangalore Genei, India) in Tris acetate bu er. Gels were stained with ethidium bromide. e resolved bands were visualized on a UV-transilluminator at a wavelength of 360 nm.

5 µl of Plasmid DNA was added to 100 µl of competent cells prepared (*E.c,,*). e vial was gently tapered and kept on ice for 20 min. e vial was incubated in the water bath at 42˚C for 2 min such that the competent cells are immersed. A er completion of 2 min the vials were quickly removed and chilled the vial on ice for 20 min. 1 ml of LB broth was aseptically transferred to the vial and incubated for 1 hour at 37˚C to allow bacteria to recover and express the antibiotic resistance. en it was plated onto the LB plate containing antibiotic (Tetracycline), X–Gal and IPTG. e results were noted on the basis of colour colonies developed on the plates.

R

Out of twenty ve crabs (*P. a , , e*) that were collected

di erent organic acids in decreasing the heat resistance of *Pae bac a* spores and the relationship between concentration of the undissociated form of di erent organic acids and decrease in heat resistance. e heat resistance of *P. a* spores was tested in distilled water at 85, 90 and 95˚C, at pH 4 and in the presence of 50, 100 and 200 m mol l^{-1} of the undissociated form of lactic, citric or acetic acid and sodium citrate or acetate. e undissociated form of organic acids was responsible for increasing the heat sensitivity of spores.

Upon the introduction of a new antibacterial into the market, the development of resistance is simply a matter of time. Hence, the knowledge of speci c resistance pathways, including information regarding molecular mechanisms in atomic detail, can provide crucial insights into the development of novel drugs. Molecular knowledge and behavioral changes must progress hand-in-hand if serious progress is to be made in the war against the development of antibiotic resistance [13].

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