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## RESEARCH ARTICLE

### ANTIBIOTIC SENSITIVITY TEST OF HYDROCARBON DEGRADING *PSEUDOMONAS AERUGINOSA*.

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Antibiotic sensitivity, Hydrocarbon degradation; *P. aeruginosa*.

#### Abstract

**Aim:** To test the antibiotic sensitivity of hydrocarbon degrading *Pseudomonas aeruginosa*, to overcome its virulence factor. **Material and Methods:** Isolation of *Pseudomonas aeruginosa* from waste water sample released from the oil refinery industry and further tested for the sensitivity test with the different antibiotics. **Results:** In decision, eight antibiotics were tested. Among them, highest sensitivity was recorded for the three antibiotics and other with least effectivity. **Discussion and Conclusion:** During assay it has been observed that *Pseudomonas* isolates found to be highly sensitive for most of the antibiotics and pertained to be safe for environmental usage as compared to those which remain in nature with antibiotic resistant features and may be lethal to the living system.

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#### Introduction: -

Even though nature has the ability to repair damage made by the creatures and by natural ways, process of keeping environment sustainable for every living creature has become the ultimate priority for human because we are not only the one who are the most evolved species available on earth but also utilizing the natural resources to the fullest; making nature unable to reposition it to the normal. In a view, human has to take some cognizant action which will add up the process of restoration in hand with the nature who is the biggest restorer available. Keeping in mind researchers across the world try to control the pollution reaching the soil, water and air by involving master class microorganisms prevalent in the nature and those harbor special features with capability of functioning in specific way by virtue of their adapted genomes. In a current study one of the known bacteria *Pseudomonas aeruginosa* has been attempted to investigate and surely this bacterium has some special features to produce the extracellular compounds recognized as Biosurfactants and by utilizing it controlling the pollution reaching to the soil and water has been represented in several recent studies.

Apart from the biotechnological potential available in the species *P. aeruginosa* their known virulence is one of the limiting factors restricted its use for many instances. Keeping in mind worker Grosso-Becerra *et al.* (2016), genetically engineered non-virulent strain of *P. aeruginosa* ATCC9027 with the operon encoded by the plasmid of the virulent *P. aeruginosa* having the genes sets involved in synthesis of this biosurfactant (rhlA and rhlB), as well as the gene that codes for the RhlR transcriptional regulator. In further studies, they reported its non-virulence in mouse model studies and also determined the potential of engineered strain in industrial mono-rhamnolipid production. In one report workers Gong *et al.*, (2015) advocated the use of gene pool of *P. aeruginosa* which

encodes rhamnolipids by constructing engineered *Escherichia coli* strains which is non-pathogenic and also able to produce the biosurfactant up to 124.3 mg L<sup>-1</sup> which was about 1.7 fold higher than normal (Gong *et al.*, 2015). Role of the *P. aeruginosa* in degradation of the hydrocarbon has well documented over the years. Now attempts have been made to check antibiotic sensitivity of *Pseudomonas aeruginosa* against antibiotics.

### Material&Method: -

#### Isolation and Identification of *P. aeruginosa*: -

The sample of waste water collected from the industrial area identified for the bacterial isolate with the ability to degrade hydrocarbon and then identified as *P. aeruginosa* NGB2, NGB3, NGB4 strain confirmed by the oil spreading technique (Anandraj *et al.*, 2010); Emulsification Activity Measurement (Ilori *et al.*, 2005) and by involving 16S rRNA gene sequencing with assigned accession number LC176070, LC176071 & LC176072.

#### Antibiotic Sensitivity analysis:-

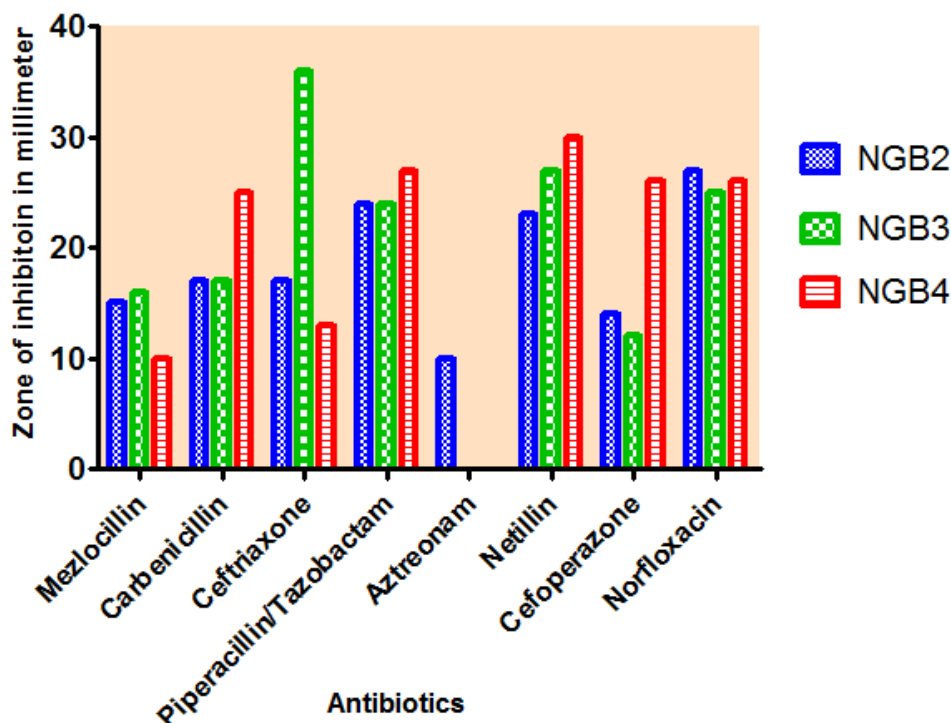
Isolated *Pseudomonas aeruginosa* strain were checked for the antibiotic sensitivity against eight antibiotics. Antibiotic sensitivity test was performed with discs of Hi-Media Laboratories Pvt. Ltd. Mumbai, India viz., Piperacillin/Tazobactam, Netillin, Norfloxacin, Mezlocillin, Carbenicillin, Ceftriaxone, Aztreonam and Cefoperazone by standard disc diffusion method. The overnight grown culture was inoculated on the solidified nutrient agar and allowed to absorb in the nutrient agar for 15 minutes. Afterwards, four antibiotic discs were placed in each plate and plates were incubated at 37°C for 24 h. After incubation developed zone around each antibiotic was measured in millimeter using the antibiotic sensitivity zone scale C (HiMedia) and culture sensitivity was categorized as sensitive, intermediate and resistant based on the CLSI chart.

### Results: -

As the *P. aeruginosa* was known for its pathogen nature, a detailed investigation has been done for the antibiotic sensitivity and during experiments if required, conditional controlling action could be taken by using the effective antibiotics as and when required. In decision, Piperacillin/Tazobactam, Netillin, Norfloxacin, Mezlocillin, Carbenicillin, Ceftriaxone, Aztreonam and Cefoperazone were tested. Among them, highest sensitivity was recorded for the three antibiotics Piperacillin/ Tazobactam, Netillin and Norfloxacin by all three *P. aeruginosa* NGB 2, NGB 3 and NGB 4 (Table No. 1/Graph No. 1). In which, NGB 2 highlighted 24, 23, and 27 mm inhibition; NGB 3 highlighted 24, 27 and 25 mm inhibition and NGB4 also showed 27, 30 and 36 mm inhibition against Piperacillin/Tazobactam, Netillin and Norfloxacin respectively. On the other hand, antibiotics Mezlocillin, Carbenicillin, Ceftriaxone, Aztreonam and Cefoperazone found to be least effective in controlling the growth of all three isolates suggesting the use of most effective antibiotics could be recommended (Table No. 1/Graph No. 1).

**Table No. 1:-**Antibiotic sensitivity profile of *P. aeruginosa* recorded against eight antibiotics.

Antibiotics	Code	NGB2	NGB3	NGB4
		Zone of inhibition in millimeter		
Piperacillin/Tazobactam	PIT100/10	24	24	27
Netillin	NET30	23	27	30
Norfloxacin	NX10	27	25	26
Mezlocillin	MZ 75	15	16	10
Carbenicillin	CB	17	17	25
Ceftriaxone	CTR30	17	36	13
Aztreonam	AT30	10	0	0
Cefoperazone	CPZ75	14	12	26

**Graph No.1 : Antibiotic sensitivity of *P. aeruginosa* producing biosurfactant****Discussion: -**

Once the identity of the *P. aeruginosa* has been assigned with number of strains, one concern remained about its pathogenicity; since despite of its utility in the environmental research and having the ability to produce proteome that capable of degrading hydrocarbon and producing biosurfactant, virulence of this species cannot be overruled. As *P. aeruginosa* is equally pathogenic to human and animals which creates doubt about its use in broad spectrum mode and needs to be control if spread or persist locally. The numbers of attempts have been carried out by the researchers worldwide to control its virulence and also advocated the use of non-virulent strain in biosurfactant production in more than ten studies (Kumar & Ting, 2016; Grosso-Becerra *et al.*, 2016; Morkunas *et al.*, 2016; Gupta *et al.*, 2016; Jani *et al.*, 2016; Quesada *et al.*, 2016; Zhao *et al.*, 2016; Rahim *et al.*, 2016; Harrison *et al.*, 2016; Hall *et al.*, 2016; Maisuria *et al.*, 2016; El-Shaer *et al.*, 2016; De & Mukherjee, 2016).

In present study, as the potential of naturally isolated local strains of *P. aeruginosa* was carried without considering its possible potential of virulence apart from biosurfactant production; keeping this in mind antibiotic sensitivity study was carried out to gauge the extent of virulence available in each strain as an indirect measure for pathogenicity and also it could be used in case of preventive action if required. During investigation, *P. aeruginosa* NGB 2,3 and 4 were considered in every antibiogram and it has been recorded that among the eight antibiotics tested, effective nature of three antibiotics Piperacillin/Tazobactam, Netillin and Norfloxacin was recorded while other antibiotics remain resistant or intermediate. The ability of these three strains for increased antibiotic resistance directed us that the mass spread of these strains could not be advisable and may be pathogenic if spread in nature without making them non-virulent. Instead by purifying extracellular biosurfactant produced and thereafter its use in degradation has been advocated.

**Conclusion: -**

In present study it has been observed that with sampling of the water and further by its microbial analysis, many strains of particular species known as *Pseudomonas* has been successfully isolated on selective media. These isolates show general prevalence in water, soil and even in human for many instances and reported for its pathogenesis, hence it is imperative to be get tested for the antibiotics sensitivity. During assay it has been observed that *Pseudomonas* isolates found to be highly sensitive for most of the antibiotics and pertained to be safe for

environmental usage as compared to those which remain in nature with antibiotic resistant features and may be lethal to the living system.

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