Analysis of expression of efflux pump (mexc2) gene in drug resistant *Pseudomonas aeruginosa*.

B. S. Pawar[1], A. A. Survase[1,2], A. S. Jadhav[1], P.H. Mahadik[3], V. M. Ghorpade[1*]

[1*]Department of Microbiology, Yashavantrao Chavan Institute of Science, Satara (Autonomous) 415001 (MH), India

[2]Rayat Institute of Research and Development, Satara 415001 (MH), India

[3]Department of Statistics, Yashavantrao Chavan Institute of Science, Satara (Autonomous) 415001 (MH), India

[*]Corresponding author:

Dr. Vilasrao M. Ghorpade, Department of Microbiology,
Yashavantrao Chavan Institute of Science, (Autonomous) Satara–415 001(India)
Mob.No.+91-9421184820
Email.ID: vilasraoghorpade@hotmail.com

Abstract: -

Pseudomonas aeruginosa. is one of most prominent type of pathogen mainly involved in most of the hospital environments and eminently involved in nosocomial infections. The epidemiology of multiple drug resistance *Pseudomonas aeruginosa* is more observed. *Pseudomonas aeruginosa* infected patient requires in-depth care and attention. They should get treated with multiple types of antibiotics at critical and higher risk. *Pseudomonas aeruginosa* shows deviation in mechanism of drug resistance to different antibiotics. In this studies *Pseudomonas aeruginosa* was found to be highly resistant to cefotaxime and ceftriaxone. In the presence of cefotaxime antibiotic regulation of gene expression was investigated by using real-time PCR.

Keywords: - Antibiotics, Multiple Drug Resistance (MDR), *Pseudomonas aeruginosa*, Gene Expression

1.0.Introduction:

Pseudomonas aeruginosa is Gram negative bacteria, *Pseudomonas aeruginosa* is a pathogen which is naturally resistance to most of the antibiotics, and majorly it is resistance to the class of number of beta lactamantibiotics. The antibiotics like aminoglycosides, piperacillin and imipenem. Other choices are also used for the treatment which includes amikacin, gentamicin and tobramycin. The effect of this drug resistance is having its impact worldwide. This Gram negative and Gram positive bacteria are causing confusing situations in treatments and their applications. About 10% of the infections which are hospital acquired are because by *Pseudomonas aeruginosa* [2][3]. Most of the deadly and life threatening infections involves *Pseudomonas aeruginosa* as a main causative agent .The most important

point of concern is the limited amount of susceptibility of *Pseudomonas aeruginosa* to most of the microbial agents it also consist ability of higher resistance to drugs [4]. This bacteria survive easily and for a longer period of time in the environment of the hospital. It has also developed an higher risk of transmission among the hospitalized patients [1]. Sometime leads to the outbreak of cystic fibrosis among the patients attending some clinical treatments over the past few years, a noticeable. Increased resistance among Gram negative bacteria is been recovered [2,3]. In room number of hospitalized patients are reported and many of them have illness in its critical stage [6]. Most of the prominent pathogens are enterococci resistance to vancomycin and *Staphylococcus aureus* resistance to methicillin, also called as vancomycin resistance enterococcus (VRE) and multidrug resistance *Staphylococcus aureus* (MRSA) infections [7]. Economic cost may be a delicate measure along with increase in number of mortality, morbidity to speculate the burden of antimicrobial resistance [8]. Higher exposure of antimicrobial agents is also major cause of higher rate of bacterial resistance in others [9]. The event of horizontal gene transfer is known to be the most important event for the wide spread range of resistance among the pathogens[10].

Pseudomonas aeruginosa much of the time shows defiance to numerous antimicrobial agents. It found in a higher reach in water bodies and it has its own interior antimicrobial resistance instrument in view of its external film, its external layer is low porousness it likewise it comprises of advanced efflux siphon framework .There are five groups of efflux siphon framework among them RND group of siphons is one from that normally comprises of three parts that incorporate an internal film drug-proton antiporter (the RND part), and OM channel-shaping protein(the OM factor [OMF]), and a periplasmic connect protein (the film combination protein). This protein joins two significant parts called MexX and MexY. These are internal layer and periplasmic protein which are encoded by operon called mexXY. The apparent OMF for this framework is the OprM. The result of the third quality of operon incodes the other three parts of the RND sort of siphon. MexAB-OprM. The conformation of the freaks that coming up short on any of the OM protein, OpmH, OpmG, Opml are aminoglycoside. The hyper vulnerability recommend that one of this can likewise the capacity with MexXY. Maybe as conscious OMF FOR EFFLUX SYSTEMMexXY changes the quantity of scopes of antimicrobials. This antimicrobials incorporates glycylcyclines, chloramphenicol, B-lactams, fluoroquinolones, macrolides, antibiotic medications, lincomycin inspite all reality that main in defiance with erythromycin, glycylcyclines, antibiotic medications and aminoglycosides. In the wild sort of cells for the most part on account of these agents may shows mexXY quality articulation . *Pseudomonas aeruginosa* is a microscopic organism which is consistently acquiring the obstruction component by mean of plasmid and periodicaly fostering the multidrug opposition all through the treatment [9]. In the current examinations, we have isolated *Pseudomonas aeruginosa* from emergency clinic conditions and attempted to examine them concerning MDR. The defiance for cefotaxime and ceftriax was tried by performing Kirby Bauer circle dissemination method. All the tests were performed by keeping the guideline institutional rules.

2.0.Materials and methods:

Isolation of *Pseudomonas aeruginosa* from the hospital environment *Pseudomonas aeruginosa* was isolated from a local hospital environment by using a cotton swab in nutrient broth. These samples were collected from the patient's skin, utensils, floor, beds and walls. The specimens were inoculated in nutrient broth tubes for 24 hours at 37°C and growth was observed identification of *Pseudomonas aeruginosa*.

The presence of *Pseudomonas aeruginosa* was performed in the microbial science research center from the clinical example, 100µl of the example was immunized on the specific medium and cetrimide agar, following 48 hours of incubation individual provinces were picked and their purity was resolved via Gram staining. Morphological characters. Susceptibility testing was finished via robotized stock testing (Neg/urine combo board; Dade Behring Institute). Development of bacterial After 24 hours detaches in presence of ceftriaxone and cefotaxime:

A 100µl of culture was immunized in 1ml of nutrient stock. Following 24 hours of brooding various groupings of anti-toxin cefotaxime in µM/ml (0, 525 and 125) were added into a test tube containing 4ml nutrient stock. The last volume is made up to 5ml by adding the 24 hours developed culture. Tubes were considered brooding at 37°Cfor 24 hours. Same strategy was continued utilizing ceftriaxone.

2.1. Isolation of RNA:

Isolation of RNA is carried out with the chomozynski and sacchi method 1987. TRIzol reagent was used in this method which carried out sequential precipitation of DNA, RNA, and protein from the sample and homogenized. RNA precipitated with isopropanol, DNA precipitated with ethanol and the protein precipitated with isopropanol. RNA was separated by performing downstream process.

2.2 Electrophoretic isolation of RNA by using Formaldehyde

The RNA sample was run into the gel for approximately 15 minutes. The gel was electrophoresed at a maximum of 5 Volts per cm of gel length (70 volts for14 cm gel). To re-circulate the running buffer the pump was turned on. The electrophoresis was continued until the bromophenol blue has run halfway down the gel (approx. 2.5 hours) shown in **Fig. 3.**

2.3. cDNA synthesis and Real-time PCR for studying gene expression.

2.3.1. cDNA synthesis Reactions

For cDNA amalgamation RNA was incubated in presence of arbitrary hexamers (Promega) and dNTPs were utilized. In the wake of chilling RNase inhibitors (Promega) were added and the response was done at 25°C and 42°C. For the inactivation of reverse transcriptase, the blend is incubated at 70°C for 15 min. what's more, the cDNA is obtained, and put away at - 20°C for additional utilization shown in **Fig. 4.**

3.0 RESULTS AND DISCUSSION

Pseudomonas aeruginosa was isolated from different hospital environments by using a cotton swab in nutrient broth medium then it was allowed to grow on cetrimide agar medium and after the development of colonies, they were confirmed by performing gram staining

Fig.1. Resistance against cefotaxime after 24 hours of incubation

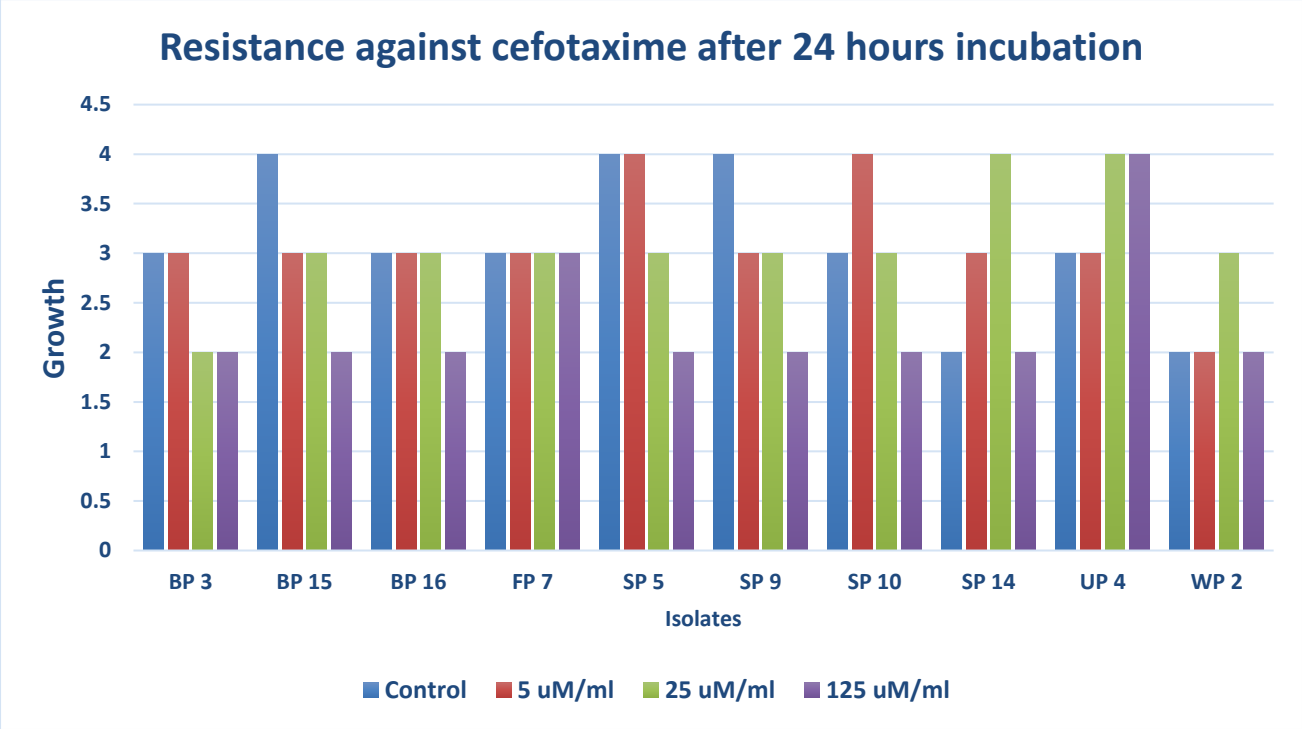
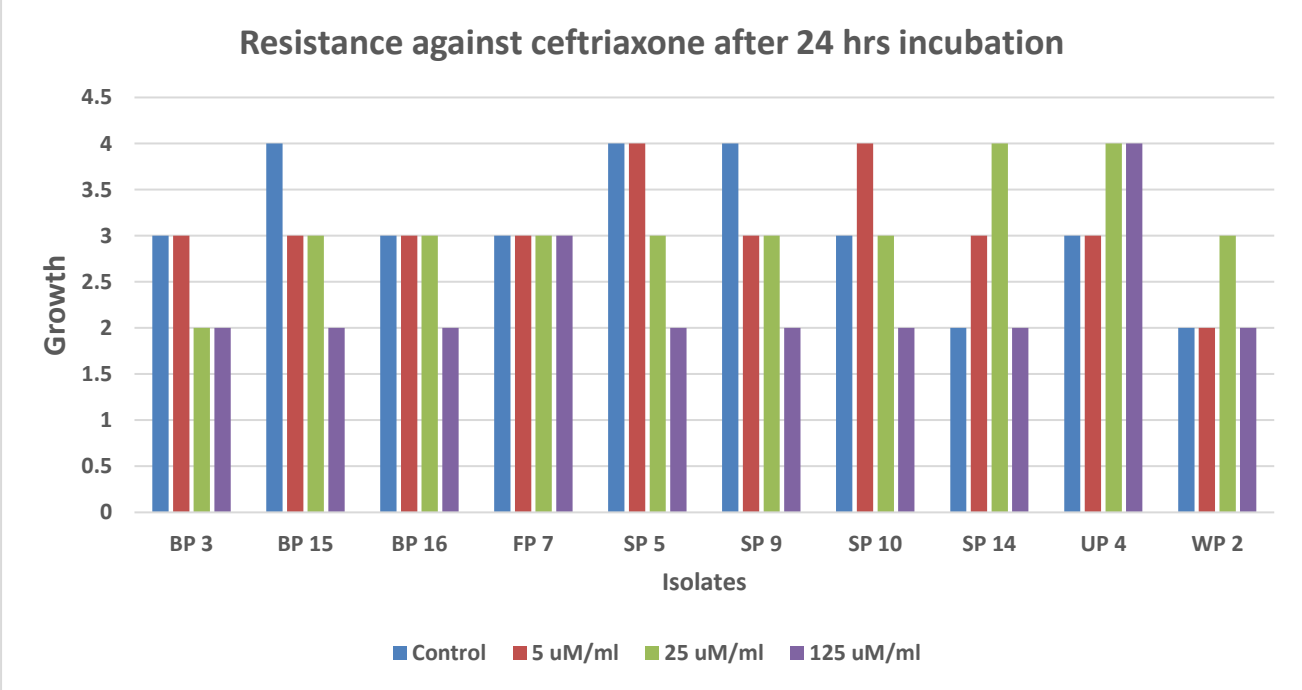


Fig. 2. Resistance against ceftriaxone after 24 hours of incubation



Pseudomonas aeruginosa clinically significant pathogens were found in hospital environments is a main cause of hospital-acquired infections *Pseudomonas aeruginosa* when incubated with different concentrations of cefotaxime antibiotic after 24 hours, it was seen that *Pseudomonas aeruginosa* grow by showing resistance against cefotaxime antibiotics in **Fig. 1.** *Pseudomonas aeruginosa* also show resistance against ceftriaxone antibiotics when used in different concentrations in **Fig. 2**. Two species of *Pseudomonas aeruginosa* named UP4 and SP5 demonstrated increased resistance when compared with other isolates.

Fig 3: Isolated RNA-A.B.C.D wells contain RNA samples isolated from organisms grown in absence of antibiotics and E, F, G, and H wells contain RNA samples isolated from organisms grown in presence of antibiotics

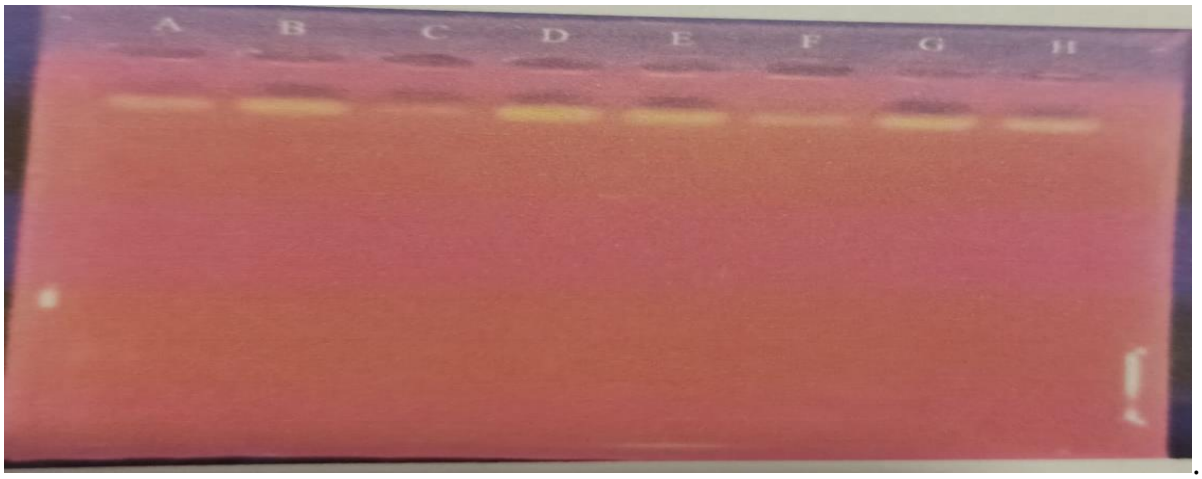
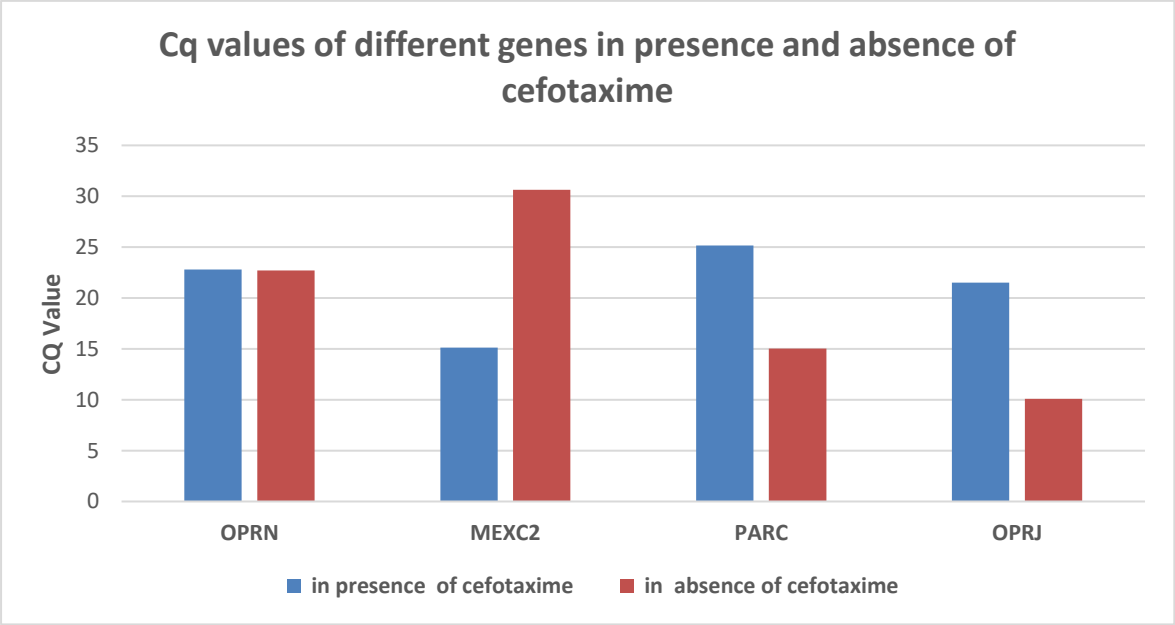


Fig 4: cDNA bands - G well contains a cDNA sample (absence of antibiotics) and H well contains a cDNA sample (presence of antibiotics)

Table 1: Cq values for different genes in the presence and absence of Cefotaxime

Genes	Cq value	
	In absence of Cefotaxime	In presence of Cefotaxime
OPRN	22.79	22.7
MEXC2	15.13	30.64
PARC	25.16	15.04
OPERA	21.51	10.08

Fig 5: Gene expression in the presence and absence of cefotaxime



RT-PCR analysis was performed to investigate the regulation of gene expression presence of antibiotic cefotaxime (125µM/ml each) in the isolate of SP5. As observed 10, when the selected isolate SPS was in the presence of antibiotics during within first 2 growth 02 folds increase was observed in MexC gene expression. The expression of O was not altered significantly with increased time of incubation. A remarkable decrease was observed in expression of PARC gene (02 folds). and OPRJ gene (02 folds).

Fig. 6. Concentration of protein in the culture broth in the absence & presence of antibiotics

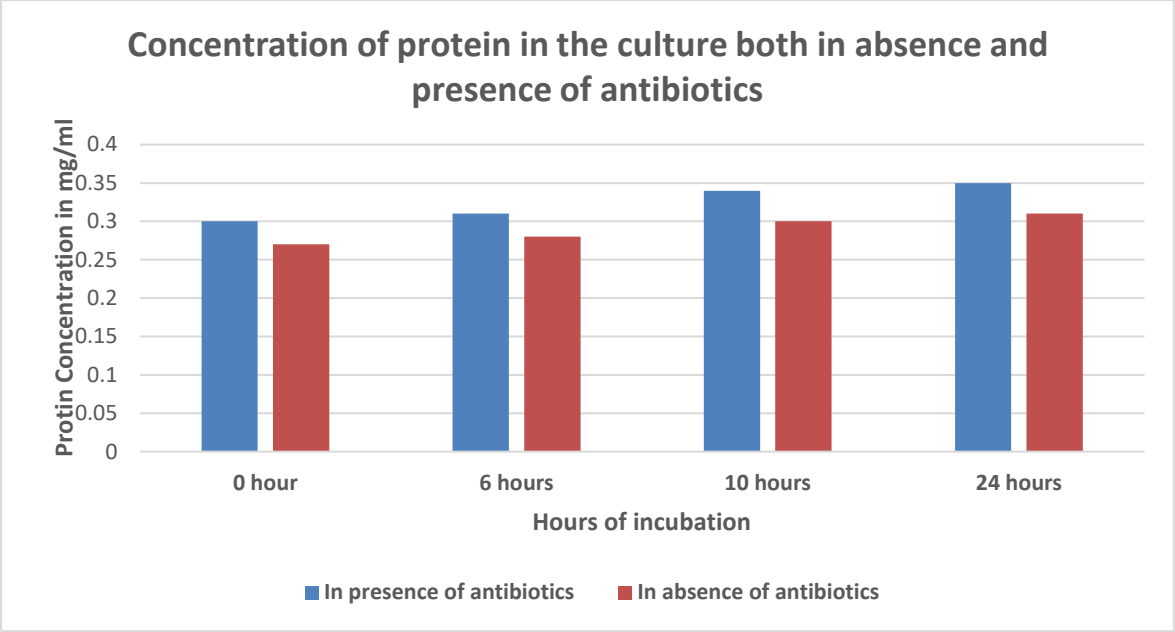
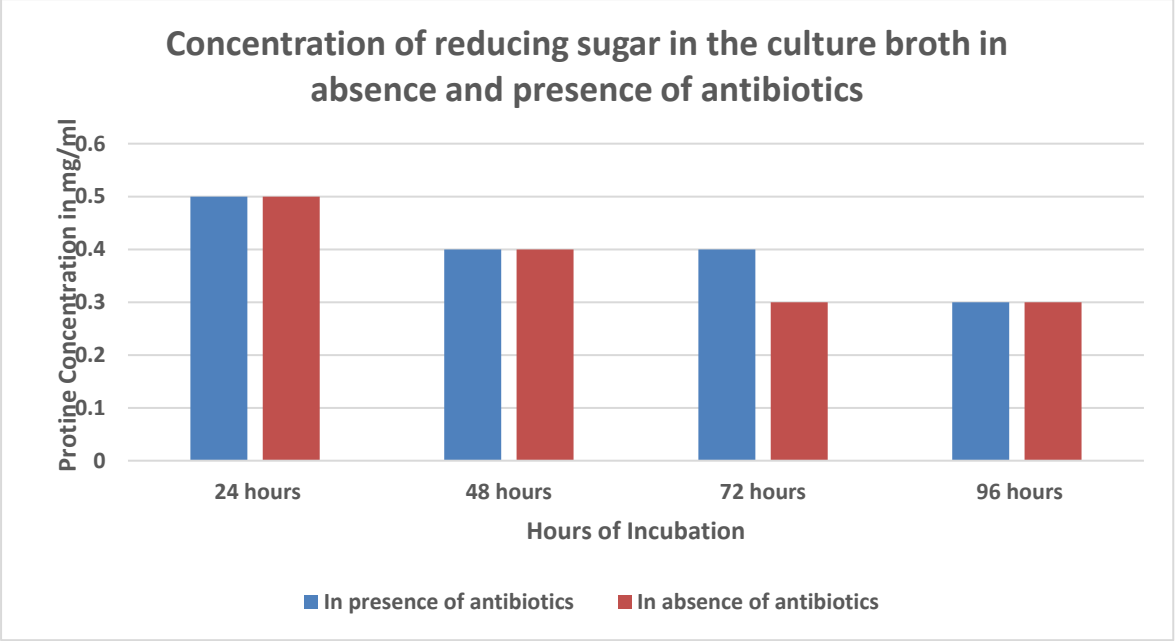


Fig.7. In the absence & presence of antibiotics concentration of reducing sugar in the culture broth



Increased concentration of protein in presence of antibiotics in the culture broth indicates more expression of genes which are responsible for the resistance observed suggesting the presence of efflux protein pump. However, no significant difference in reducing sugar utilization was observed by the culture in the presence or absence of antibiotics.

CONCLUSION

The present investigational study reveals that the *Pseudomonas aeruginosa* is capable tolerate high antibiotic concentrations. This may be because of the efflux pumps that exist in *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa.* is a common pathogen it is important to know antibiotics which can inhibit growth without side effects on the human being. The present study helps to design good antibiotics against them for the betterment of human health.

AUTHOR CONTRIBUTIONS

Each of the writers has made significant donation to the consensus and data, acquirement of information, examination and the understanding of the information; participated in drafting the creation of article and changing it fundamentally for significant and scholarly substance; All of the writers were consented to submit it to the ongoing diary; every one of the writers gave the last approval of the variant of the structure to be distributed; and consented to be at risk for the every one of the parts of the current work. The creators are all qualified to be a creator according to the global panel of clinical diary editors (ICMJE) necessities and rules.

FUNDING

The present research work is financially supported by State Project Directorate (SPD), Rashtriya Uchchtar Shiksha Abhiyan (RUSA), Maharashtra and MHRD, Govt. of India under the major disquisition of research project.

CONFLICTS OF INTEREST

All of the authors report no monetary or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study doesn't involve the experimentation on any animal creature or human subjects.

PUBLISHER'S NOTE

This journal remains neutral applicable to jurisdictional claims in the published institutional cooperation.

DATAAVAILABILITY

All the data gained during the study are presented in this manuscript. Any further added inquiries for additional for further more information are available up on request from the corresponding author

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