EFFICACY OF PYOCYANIN, EXTENDED-SPECTRUM BETA-LACTAMASE AND HCN PRODUCTION IN PSEUDOMONAS *AERUGINOSA IN* CLINICAL SETTINGS – AN EMERGING THREAT

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ABSTRACT :

Emergence of multi drug resistant bacterial strains is a growing global concern and in particular strains of *Pseudomonas aeruginosa* acquires various significant virulent characters frequently and they are always versatile than other groups of bacteria in acquiring drug resistant mechanism. Cyanogenesis, Pyocyanin and extended spectrum of beta lactamase (ESBL) production makes this bacteria more virulent than other pathogens in the current scenario. Hence the study is sought for the detection of cyanogenesis and ESBL production among the clinically recovered isolates. The results exposed that among the isolated *Pseudomonas aeruginosa* strains 17.64% produced high and 35.2% produced low and 47% with no cyanide production, 58.8% of the strains produce pyocyanin a phenazine compound, ranging from (3.02 - 9.38µg/mL) Further its antibacterial activity also were estimated during the investigation. The pigment produced significant antibacterial activity against other clinical pathogen. 67.7% were ESBL producers which indicates that the organism should be given more attention during treatment and other clinical studies. The ESBL confers more drug resistant characters to the bacterium. The result also concluded that treatment for *Pseudomonas aeruginosa* infections should include cyanide detoxifying agent and based on that, drug should be chosen for effective treatment.

Key words: HCN production, ESBL, MAR index ,*Pseudomonas aeruginosa*, Pyocyanin.

1.INTRODUCTION:

Pseudomonas aeruginosa is a gram negative, aerobic rod sahped bacterium and well known for its opportunistic pathogenicity being adopted as an ubiquitous organism which survives in hospital environment favoring the spread of infections among immune compromised and hospitalized patients(1).The organism cause various infections like pneumonia, keratitis and folliculitis in both healthy and immune compromised individuals (2). The ability of *P.aeruginosa* to cause infection is further exacerbarated by its high degree of resistance to various antibiotics making pseudomonas infections a great challenge to treat(3). Many virulence factors like enzymes, efflux pumps, exo polysaccharide, pili, exotoxin A, lipo polysaccharide, pigments, lipase, haemolysin, histamine, exoenzyme S, leukocidin, rhamnolipids, horizontal gene transfer mechanism and bio film production were utilized by this organism and all of the virulence factors contributed to the bacterial invasion and toxicity(4). In addition very recently *Pseudomonas* produce extended-spectrum beta-lactamases (ESBLs) were discovered which further confers resistance at various levels to expand its spectrum of resistance to Cephalosporin's and other used drugs. ESBL producing bacteria mostly escape from the routine disk diffusion susceptibility test, leading to an inappropriate use of antibiotics and treatment failure(5).

Cyanide is always considered as a potent toxin when exposed to humans which leads to fatal condition. It acts by binding with several metallo enzymes, but it inhibits cytochrome-c oxidase(complex IV of the mitochondrial respiratory chain) which appears to be responsible for toxicity. Mostly the organs which consumes high amount of oxygen are highly sensitive to cyanide particularly heart and brain which utilizes high oxygen are sensitive to cyanide. (6).

P.aeruginosa through the enzyme HCN synthase encoded by *hcnABC* synthase gene cluster produce high amounts of cyanide when glycine is incorporated in the medium. Glycine is decarboxylated to hydrogen cyanide(HCN)(7),(8),(9). The presence of cyanide in sensitive terminal oxidase expresses rhodanese which further detoxifies cyanide by converting it to thiocyanate favoring the organism to resist cyanide.(10). *P. aeruginosa* normally infects wounds in patients with burns, and cyanide may contribute to the pathogenicity of the bacterium in clinical condition. (11).

Pyocyanin production extracellularly during exponential phase as a secondary metabolite of most of the *P.aeruginosa* is a phenazine derivative blue-green pigment. These are heterocyclic compounds produced naturally and substituted at different points around their rings by different bacterial genera particularly expressed in *P.aeruginosa.* The redox-active phenazine compound has the ability to destroy mammalian and bacterial cells through the generation of reactive oxygen intermediates(12),(13).

Mainly the virulence is present in the blue pigment pyocyanin that has deleterious effect on number of prokaryotic organisms, possibly due to the formation of hydroxyl radical (14). Moreover Pyocyanin (*N*-methyl-1-hydroxyphenazine) has antibiotic activity against a wide variety of microorganisms (15),(16). Therefore the pathogenic potency of this organism is not determined by single virulence factor, but the whole array of factors contributes towards pathogenicity. Hence the Current study was primarily focused on the severity of *Pseudomonas aeruginosa* infections, which pose a major problem in human health.

2.MATERIALS AND METHODS

2.1.Isolation source and identification of *P.aeruginosa*

The study was examined with 102 clinical samples comprising of Burns (n=3), Pus (n=42), wound swabs(n=20), Urine(n=20) and sputum(n=17) from various clinical settings in and around Puducherry, India. *Pseudomonas aeruginosa* were presumptively identified after aerobic culture on Cetrimide agar (Hi Media, India) with an incubation time of 24 h at 37ºC as per standard methods of identification (Williams and Wilkins, 1994). Isolates were further categorized based on their distinct biochemical and pigment production characteristics(17).

2.2.Molecular identification of *P.aeruginosa* and phylogeny reconstruction

The colony PCR protocol was adopted for identifying the genus and species level identity of the pure cultured isolates recovered clinically and they were presumed to be *P.aeruginosa*. The isolated culture was subjected to 16s rRNA sequencing and identification using Universal 27F Forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R Reverse 5'-GGTTACCTTGTTACGACTT-3'primers. The resultant amplified product (1400 bp) were purified and re-subjected to 7yhn Sequencing using specific primers (785F 5' GGA TTA GAT ACC CTG GTA 3' and 907R 5' CCG TCA ATT CCT TTR AGT TT 3') (Weidner *et al.*, 1996). Sequencing were performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA) and the sequenced products had been resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA).

Sequencing results were subjected to BLAST analysis and the phylogenetic was constructed using MEGA 6 (Tamura *et al*., 2013). In the tree the numbers at the nodes indicate the levels of the bootstrap support [high bootstrap values (close to

100%) meaning uniform support] based on a neighbour-joining analysis of 1,000 re-sampled data sets. The bootstrap values below 50% had not indicated. Bar 0.005 substitutions per site.

2.3.Determination of Multiple Antibiotic Resistance

Antimicrobial susceptibility testing of the recovered *P.aeruginosa* isolates were performed by Kirby-Bauer agar diffusion method. Commercially available (Hi-Media) antimicrobial discs of Amikacin (Ak 30µg), Gentamicin (G 10 µg), Meropenem (Mr 10µg), Ceftriaxone (Ci 30µg), Ampicillin (A 10µg), Penicillin G (P10 µg),Ciprofloxacin (Cf 5µg), Tetracycline (T30µg), Chloramphenicol (C 30µg), Streptomycin (S 10µg), Azithromycin (At 15µg), Clindamycin (Cd 2µg), Polymyxin B (Pb 300U) were used on Mueller Hinton agar(MHA, Hi-Media) to test the susceptibility. Anti bio gram (sensitive or resistance) profile was obtained as per the protocols designed by Clinical and Laboratory Standards Institute Guidelines. (2011/M100S21; http://clsi.org; Clinical and Laboratory Standards Institute 2011).

2.4.Multiple antibiotic resistance (MAR) index of *P. aeruginosa*

The MAR index to a single isolate is defined as a/b, where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the number of antibiotics to which the isolate was exposed. MAR index value higher than 0.2 was considered to be originated from high risk sources of contamination (20).

2.5.Detection of Cyanogenesis in *P. aeruginosa*

For detection of cyanide producing ability the isolated *P. aeruginosa* strains were streaked on King's B medium amended with glycine at 4.4g/ L(substrate of cyanogenesis) in which sterile filter paper saturated with picric acid solution (2.5 g of picric acid; 12.5 g ofNa2CO3, 1000 mL of distilled water) was placed in the upper lid of the Petri plate for the detection of cyanogenisis . The plates were incubated at 28ºC for 48 hours with complete sealing of the lid with parafilm. Positive reaction for cyanide production was ascertained by observing the change in the colour of the filter paper from yellow to light brown, brown or reddish-brown, and the results were grouped as weak (+), moderate (++) or strong (+++) reaction for HCN production(21).

2.6.Production of Pyocyanin

All the isolates *P. aeruginosa* strains were cultured on King's A medium and incubated at 37°C for 48 hours then incubated at room temperature for 7days. The strains that showed greenish blue pigment production were considered positive for pyocyanin production (22).

2.7.Quantitative Assay for purification of Pyocyanin

Quantitative assay for the production of pyocyanin were conducted by Essar *et al*.(23) and later modified by Saha *et al* (24). The characteristic green pigmented colonies were grown in broth culture, centrifuged and the pigment was extracted using chloroform extraction method using sequential acid and neutral water. By following extraction procedure blue color solution was obtained in chloroform and turned into pink upon addition of 0.1N HCL were observed which confirmed the production of pyocyanin pigment by the isolates.

Concentrations expressed as micrograms of pyocyanin produced per ml of culture supernatant were calculated by using an extinction coefficient at 520 nm of 17.072. After completion of all the steps , pH was adjusted to 7.5 with addition of 0.1M NaOH and needle like crystals were also observed in the chilled solution after 2hours.

2.8.Antimicrobial activity of the purified pigment

The antibacterial activity of the purified pigment were monitored against clinical bacterial pathogens *Staphlyococcus aureus*, *Shigella* sp, *E.coli*, *Serratia* sp, *Klebsiella* sp and *Salmonella* sp. following agar well diffusion method in Mueller-Hinton Agar (25). First the prepared media were seeded with the test organism (OD equivalent to 0.5 McFarland), spread in

the petri plate, solidified and wells were prepared in the agar medium. Then 100 µL of extracted pigment solutions from the highest pyocyanin strains were used for further investigation. The extract was dispensed in the wells of the agar plate and incubated for 24 hours at 37ºC. After incubation, the diameter of zone of growth inhibition was measured to determine the antimicrobial activity of the test agent.

2.9.Detection of ESBL by double disc diffusion synergy method

All the *P. aeruginosa* isolates exhibited resistance to the antimicrobial discs which were further investigated for the ESBL production by modified double disc synergy Test (MDDST). Amoxicillin-clavulanate (20/10 µg) along with cephalosporins; 3GC-cefotaxime, ceftriaxone, and Aztreonam the results were interpreted according to Collee et al. 1996 and Drieux et al. 2008. The isolates were inoculated on Muller –Hinton Agar plate As per Kirby-Bauer method. The Augmentin disc (amoxicillin-clavulanate) was placed in the center of the plate; 3GC-cefotaxime, ceftriaxone, and Aztreonam discs were placed 15mm apart from center disc. The zone of inhibition of 3GC-cefotaxime, ceftriaxone, Aztreonam and augmentin discs were compared after 16-18 hours of incubation at 35°C. The inhibition zone between 3GC-cefotaxime, ceftriaxone, Aztreonam and augmentin disc was considered to be isolates producing extended spectrum β- Lactamase.

3.RESULT AND DISCUSSION

Two types of colony morphology were observed during the bacterial growth on Cetrimide Agar viz.,colonies with green pigmentation 10 (59%) and colonies without green pigmentation 7 (41%). Among these isolates 8 (19.04%) were from Pus, 3(100%) from burns and 3(15%) from wound swabs and 1 (5.86%) isolate from sputum respectively. The result claimed that *Pseudomonas* were commonly found in all types of clinical samples which are frequently reported worldwide. The present bacterial incidence were quite similar with earlier findings where *Pseudomonas* sp in burns cases (18.18%) and wounds (6.25%)(26) were documented followed another report where 55.35% present in pus and 7.74% in wounds(27).

The prevalence rate and the wide distribution of *P. aeruginosa* in the clinical samples may vary from one hospital to the other as each hospital have a different environment and facility associated within. All the isolates were presumptively identified as *P.aeruginosa* based on conventional phenotypic methods.

3.1.Molecular identification of recovered *P.aeruginosa* strains

Based on the biochemical characterization of the isolates recovered by enrichment methods, a few strains were presumably identified as *P. aeruginosa.* However, a molecular identification was under taken to confirm the genus or species level of the selected isolates. Among the total 17strains recovered from the different clinical samples, the strains PsADMC01, PsADMC02, PsADMC003, PsADMC004, PsADMC006, PsADMC007, PsADMC008 and PsADMC009 were subjected for molecular identification, the results indicated that they all belonged to *P. aeruginosa* and further they were deposited in GenBank and the following accessions were obtained MK598324, MK598327, MK598330, MK598331, MK598332, MK598333, MK598334, MK598336 that corresponds to the strains PsADMC01, PsADMC02, PsADMC003, PsADMC004, PsADMC006, PsADMC007, PsADMC008 and PsADMC009 respectively.

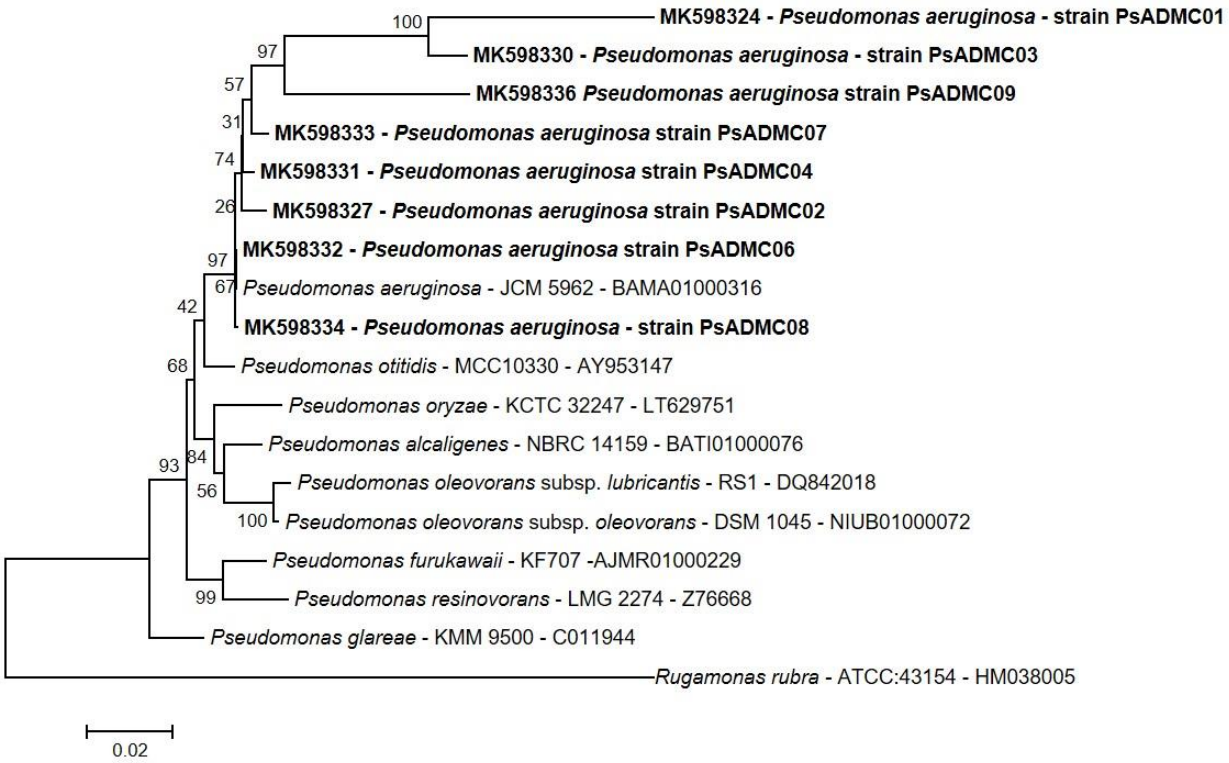

Fig. 1. Evolutionary relationship among the strains recovered in this study were reconstructed using 16S rRNA genes, the strains obtained in this study were shown in bold and the strain ATCC 43154 of *Rugamonasrubra* was used as an out group to root the tree, Scale bar represents 0.02 nucleotide substitutions per nucleotide position.

3.2.Multiple Antibiotic Resistance

The resistance/susceptibility profile of *P. aeruginosa* to the drugs tested varied among the isolates were investigated.

All the *Pseudomonas aeruginosa* isolates were completely resistant to penicillin G, Ampicillin, Tetracycline, Chloramphenicol and Clindamycin(100%) followed by high degree of resistance displayed against Polymyxin B(82.60%). For Azithromycin and Ceftriaxone the level of resistance strains were 69.56%. Meropenem resistant strains were found to be 60.86%. Moderate level of resistant been noticed for ciprofloxacin and Amikacin(43.47%) respectively. Gentamicin (39.13%) and Streptomycin (26.08%) were also identified during the study.

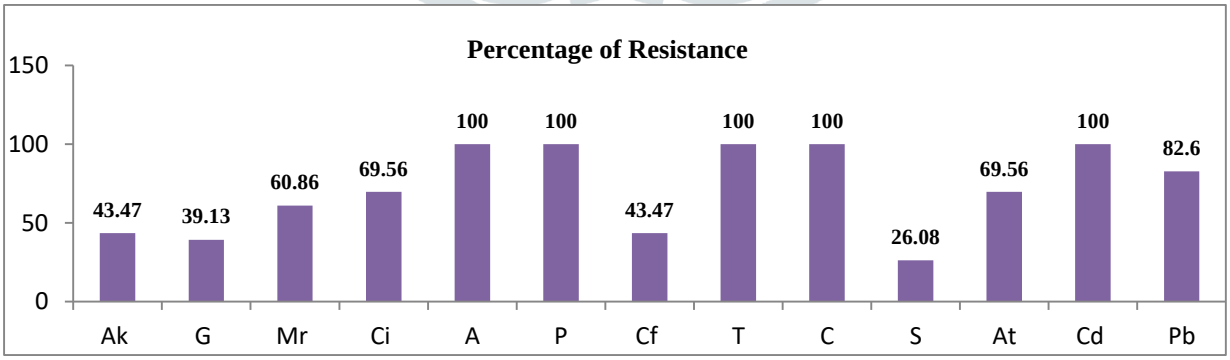

Fig .2 Resistant profile of *P.aeruginosa* strains

There has been an alarming elevate in resistance to different anti-pseudomonal drugs specifically among hospital strains world-wide (28).The present investigation results were collaborating well with earlier report of of Saha *et al.*, 2005 where the *P.aeruginosa* were completely resistant to Penicillin, Ciprofloxacin, Tetracycline, Clindamycin, Choloramphenicol and

Polymyxin B(24). High degree of Gentamicin resistance (68%) were reported in earlier report(27) but low level of resistance of Gentamicin were observed from our study.

Complete resistance to Ampicillin (100%) was also chronicled by Sivanmaliappan and Murugan, 2011(29). Resistance to quinolones like Ciprofloxacin ranges from 50–60% and Azithromycin (53.8%) were observed in the study conducted at Nigeria(30). A much higher resistance to Cephalosporin, Ceftriaxone of 86.85% and 93.9% had been reported in studies accomplished in India(31)and Nepal(32). Similarly sensitivity towards Ceftriaxone also reported from Andrapradesh, India(33)

A low level of resistance towards Amikacin(34.3%) and high level of susceptibility(65.7%) to the same drug were observed in the preceding study conducted by Saha *et al*., 2005 and the study justifies the current report as resulted in the same pattern. Highest Carbapenem resistance rate for Meropenem was about 65.52% among the *P.aeruginosa* isolates from clinical samples and the same was also noticed in a past study(34). Meropenem 83.5% sensitivity were also documented in a previous report of Al-Kabsi *et al.*,2011 should also be taken into consideration(35). In a collaborative study of Nagamani *et al.*,2011 moderate resistance pattern had been analyzed, the average resistance found for Gentamicin and Amikacin was 61.91% and 42.2% which supports the present study (36).

There are several factors responsible for the prevalence of multidrug resistant *P.aeruginosa* which includes an existence of drug modifying enzymes especially for aminoglycosides(37). Adenyltransferases allied with aminoglycoside resistance in *P. aeruginosa* include ANT (streptomycin resistance) and ANT(4)-II (Amikacin, have been reported and are encoded by genes present in the chromosome and/or on plasmids of amikacin-resistant clinical isolates.(38).

The other reason for resistance exhibited by bacteria is due to frequent and misuse of drugs even without the onset of bacterial infection. Moreover antibiotic resistance may also be transferred from other bacteria through horizontal gene transfer mechanism(39).Similarly resistance to Carbapenems may crop up due to complex interactions of several mechanisms including production of carbapenemase, surplus production of efflux system and loss of outer membrane porins (40).

3.3.Multiple Antibiotic Resistance Index(MAR)

MAR index was calculated for all the MDR *P.aeruginosa* strains and a graph illustrating the MAR index of all the isolates were shown in the Fig.3

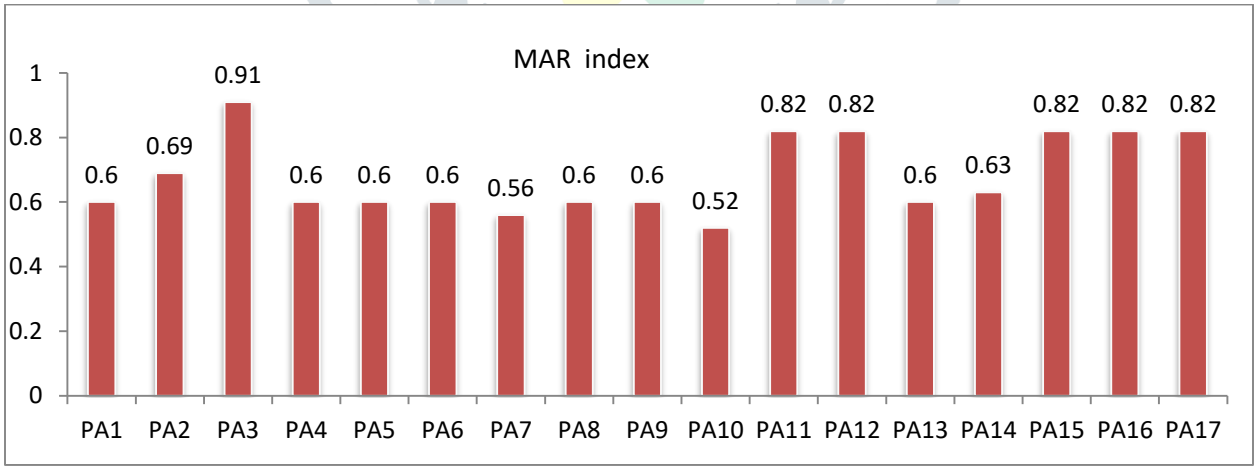

Fig.3. MAR index of *P.aeruginosa* isolates

The appearance of MAR index of the *P.aeruginosa* isolates in this current investigation range between 0.56 to 0.82 which indirectly denotes that all the isolates were multi drug resistant and these isolates were exposed to an environment of high-risk contamination from a region or area where there is high antibiotic use.(20), (41). A contemporaneous study proffer that the antibiotic usage must be analyzed by distinguishing colonization from infections and further the treatment might proceed to clinical confirmed infections and not merely the colonization.

A keen observation and strict efforts should be taken to maintain the environment under control and antimicrobial use may control the invasion of this organism in hospital environment. Regular checking of antibiogram of hospital pathogen by the infection control team is suggested to keep clinicians updated on the proper empirical treatment of such rapidly evolving resistant pathogen.

3.4.Hydrogen cyanide Production

P. aeruginosa synthesizes hydrogen cyanide as its secondary metabolite by the action of enzyme cyanide synthase encoded by*hcnABC* genes(7). Cyanide production in *Pseudomonas* was reported in 1960s especially in burn wound infections and its pathogenic role was undetermined(11). But recent investigation demonstrated that cyanide production accomplished under low oxygen conditions and it was commonly found in patients suffered from cystic fibrosis lung infections(42).

With reference to cyanide production, in the effort of present investigation 17.64% of the *P.aeruginosa* produced high and 35.2% (6) produced low and 47% with no cyanide production were observed. Similar report were also seen in the primitive study conducted at California(43).

Our findings confirmed the previous reports of Goldfarb and Margraf, 1967(11) who experimentally observed the production of HCN in *P.aeruginosa* isolated from burn and wound and similar observations were done by Ryall *et al.*,2008 from sputum(44).

The association between the cyanide production in *Pseudomonas* pathogenicity remains unclear but it may dwell with selective advantage in the ecological niches and plays its unique pathogenic role. By possessing active detoxification enzyme mechanism(rhodanese) synthesis of terminal oxidase in respiratory chain were insensitive to cyanide, the organism may escape from the toxic effects of cyanide(10),(45). While in clinical treatment it is important to understand the role of cyanide producing *Pseudomonas* in pathogenesis and the treatment may also include a cyanide detoxifying agent.

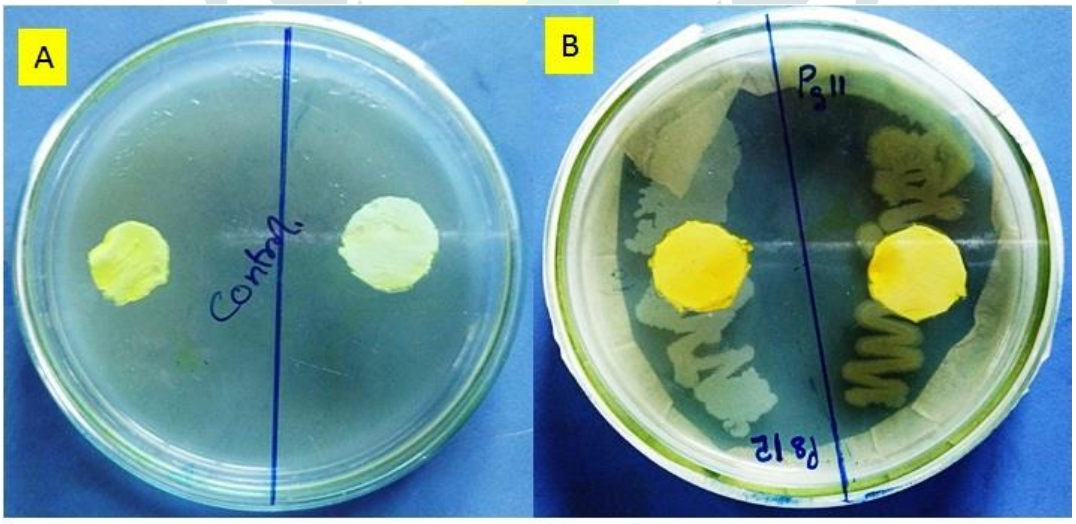

Fig.4. HCN production of *P.aeruginosa* isolates

3.5.Purification and characterization of the pyocyanin produced by *Pseudomonas* strains:

The 10 isolates of *P.aeruginosa* with green coloration were further cultured on King's A medium for augmentation of pyocyanin and all produced bluish green coloration after vigorous pigmentation within the duration of incubation period. Usually *P aeruginosa* produce different kinds of pigments pyocyanin (blue green) pyomelanin (light-brown), pyoverdin (yellow, green and fluorescent) and pyorubrin(13). The current extraction procedure resulted a blue color which was observed

in chloroform that further turned into pink color upon addition of 0.1 N HCl confirmed the production of pyocyanin soluble in chloroform and resulted in blue colour. (46). It is considered as a resonance hybrid of the mesomeric forms of N-methyl-1-hydroxyphenazine and is capable of undergoing a two-electron reduction to a colourless product, leukopyocyanin. It can exist either in oxidized form. The reduced form of pyocyanin was unstable and rapidly reacted with molecular oxygen. The pigment is wine colured at acid condition because of the basic property of one of the nitrogen atoms and blue at alkaline reaction(13). The amount of pyocyanin concentration estimated during the investigation were illustrated in the following graph Fig.4

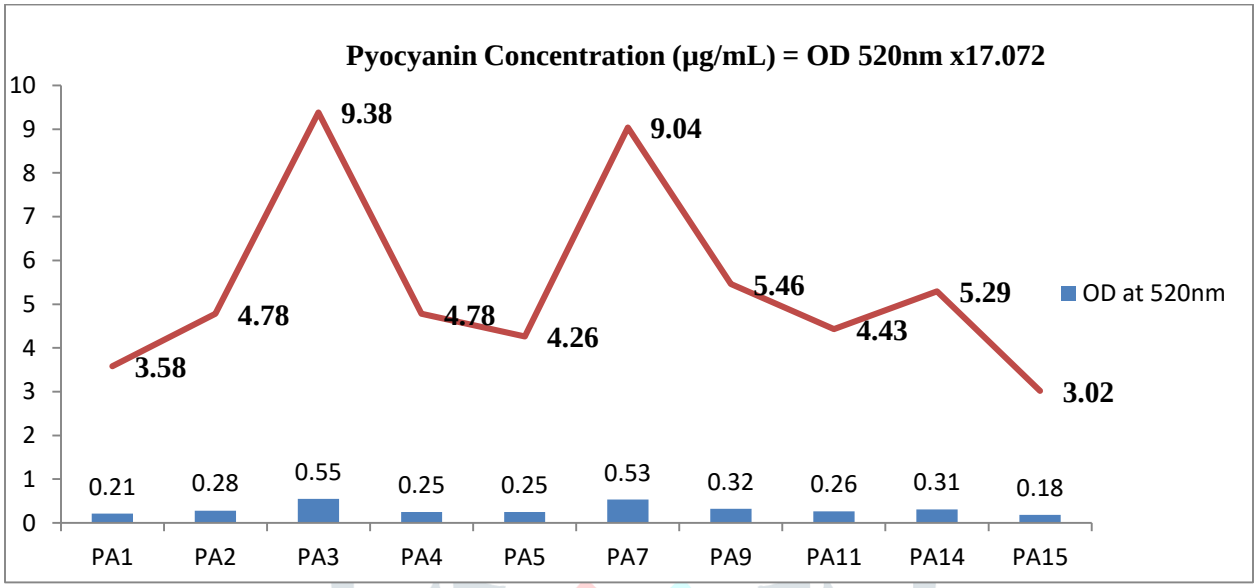

Fig.5. Quantitative assay of Pyocyanin Production

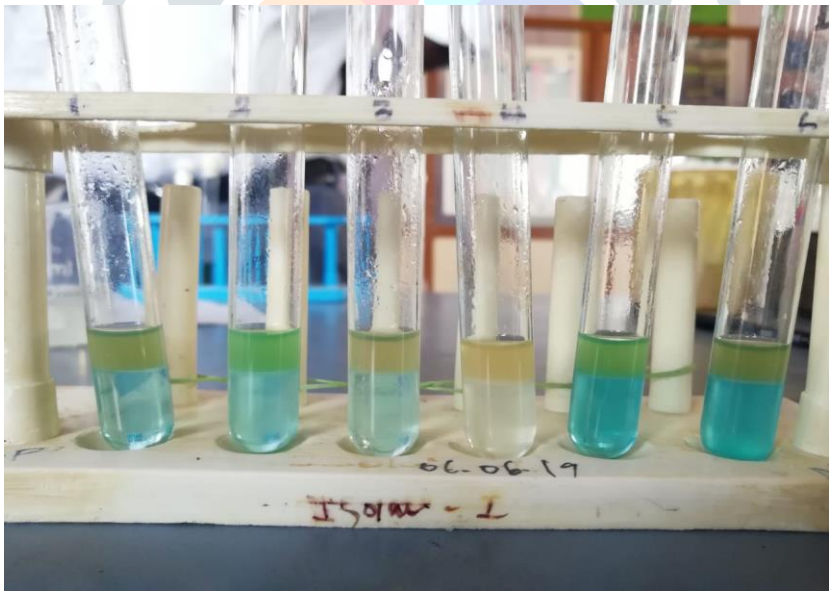

Fig.6.Blue color formation(Pyocyanin)after addition of Chloroform

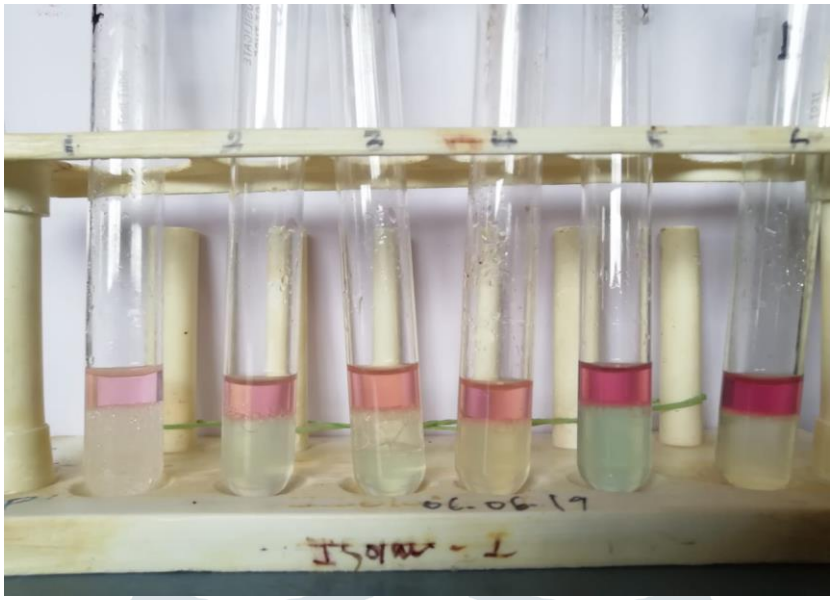

Fig.7.Pink color formation (Pyocyanin) after addition of 0.1N HCL

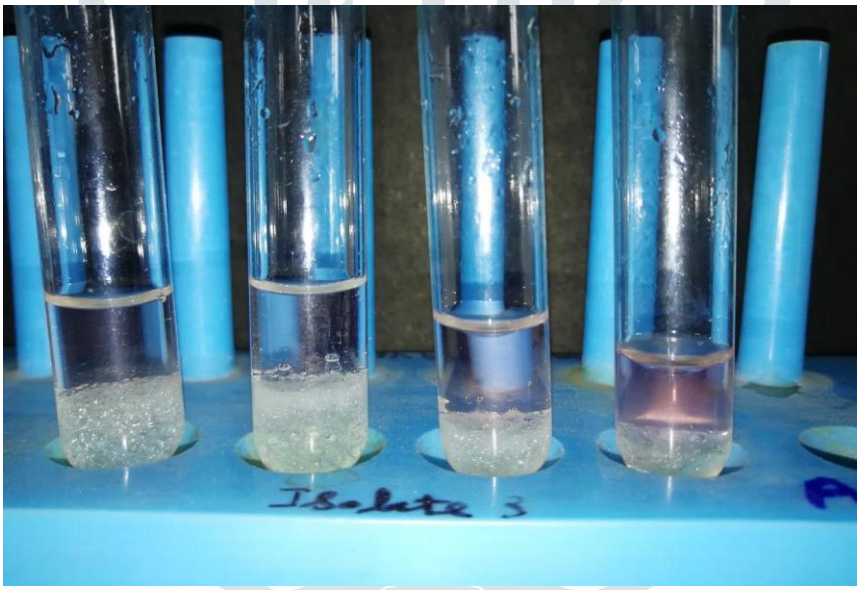

Fig.8 Crystal structure of Pyocyanin

In the present pilot study all the isolates produced significant amount of pyocyanin pigment in which PA1, PA2, PA3, PA4, PA5, PA7, PA9 and PA11 were identified from Pus samples and the remaining PA14 and PA15 were from wound samples. A very high density of Pyocyanin were estimated in PA3(9.38 µg/mL) and PA7(9.04 µg/mL) and both were obtained from Pus samples. In an earlier prospective study in Egypt conferred the high amount of Pyocyanin were regained from *P.aeruginosa* of human urine than the pigments retrieved from *P. aeruginosa* from rice cultivated soil (47). While a high quantity of pigment ascertained from Sputum(12.069 µg/mL) isolates were estimated by Ra'oof and Latif,2010(48) which correlates the findings in the study.

3.6.Antimicrobial activity of the purified pigment

The antibacterial activity of the extracted and purified pigment was challenged against clinical bacterial pathogens *Staphlyococcus aureus*, *Shigella* sp, *E.coli*, *Serratia* sp, *Klebsiella* sp and *Salmonella* sp. The results of present experimental study agreed with most published researches that showed antibacterial effect of pyocyanin on several pathogenic bacteria (Table.1)

Table.1. Antibacterial activity of purified pigment against clinical pathogens

Culture No	Zone of inhibition (mm)					
	Staph.aureus	***Salmonella sp***	***E.Coli***	***Serratia sp***	***Shigella sp***	***Klebsiella sp***
PA1	14	31	22	26	10	18
PA2	12	33	Nil	14	Nil	22
PA3	11	13	28	14	8	22
PA4	Nil	22	18	12	24	16
PA5	Nil	20	22	20	28	18
PA7	13	34	13	18	22	5
PA9	16	12	20	13	10	12
PA11	Nil	28	10	14	22	11
PA14	20	20	21	21	10	12
PA15	13	16	20	12	8	12

The present study demonstrated that the purified pyocyanin extracted from the *P.aeruginosa* isolates upon investigation against the tested clinical isolates had considerable antibacterial activity. Pyocyanin has greater antibacterial effect on gram negative pathogens Salmonella typhi, *P. mirabilis*, *E. coli*, *Acinetobacter sp*. were denoted by several studies (49). In general the pyocyanin is a phenzine compound which posses antibacterial bacterial effect on other bacterial species(50).

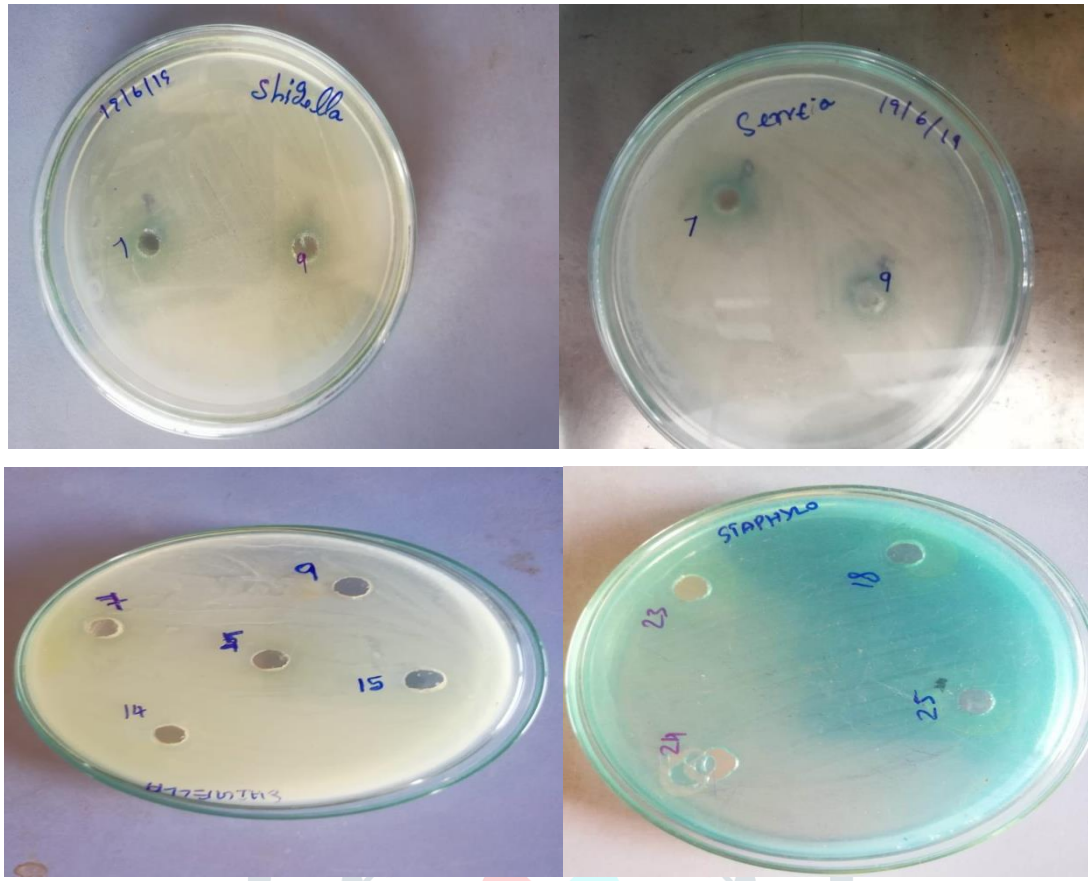

Fig.9 Antibacterial activity of Pyocyanin against clinical pathogens

3.7.ESBL production

ESBL production distinguish the bacteria from other bacterial pathogens because the ESBLs are encoded by various genes situated either in chromosome or plasmids which contributes resistance to spectrum Cephalosporins(ESCs), such as cefotaxime (CTX), Ceftriaxone, Ceftazidime (CAZ) and the Monobactam Aztreonam(ATM). This kind of bacterial pathogen bears an unique challenge to medical professionals and scientist engaged in finding new antibacterial products. ESBLs are most commonly found in *Klebsiella pneumoniae* and *Escherichia coli* and have been detected in *P. aeruginosa* recently at low frequency(51). 67.7% of the isolates were found ESBL producers during the current investigation in which 59% of them produced visible pigments. Though there is no correlation between pigment production and ESBL activity, ESBL activity will exceedingly challenge the treatment. In India, prevalence rate of ESBLs ranging from 28% to 84% has been reported from various parts of the country. Bakshi *et al*., 2013 reported high prevalence of (50%) ESBL among *P. aeruginosa* at Patiala (Punjab), South India. However higher incidence of ESBL among *P.aeruginosa* (42.31%) has been reported by researchers at AIIMS, New Delhi(52) and even higher rate to the tune of 64% has been reported by (53)Mathur *et al.*, 2002 from South India.

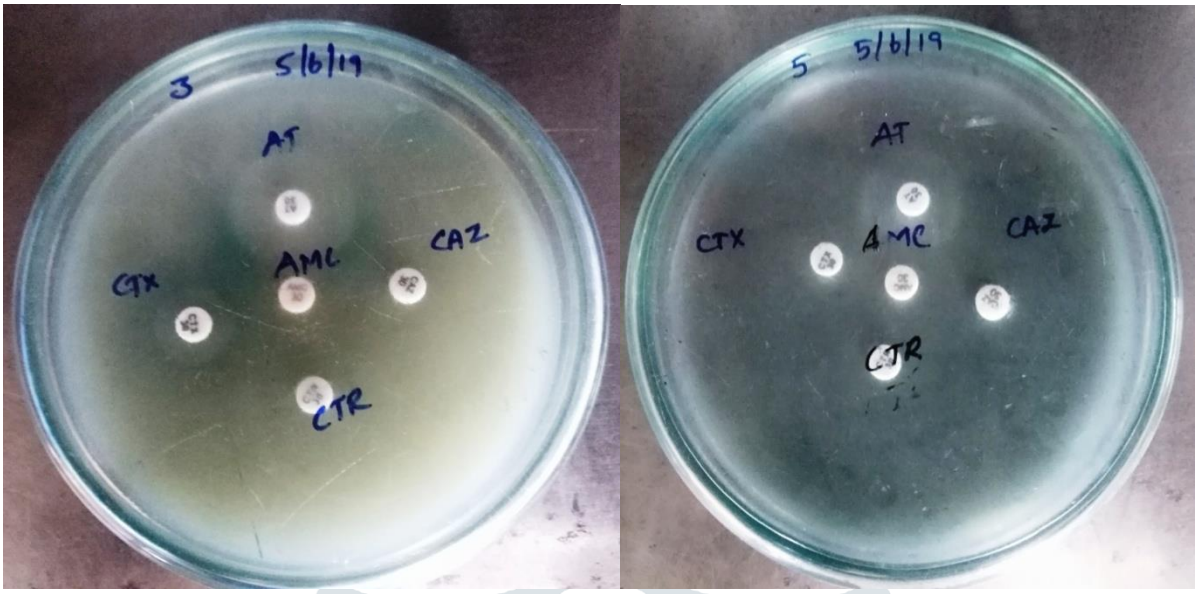

Fig.10 ESBL production of *P.aeruginosa* strains

4.CONCLUSION

On the basis of preceding study *P.aeruginosa* yet again confirmed with its more potential virulence from clinical settings. Our assessment and the available substantiation suggests that though there may be many virulence factors which contribute to the prevalence of MDR *P.aeruginosa* in the human environment, Cyanogenesis in *P. aeruginosa* may destruct cellular functions of lungs and the long term damage of lung cells may results in chronic infections which should be taken into account seriously during an respiratory infections. A glance on ESBL production should be done before initiating any antibiotic therapy will channelize correct choice of drug. Pocyanin production during respiratory and other infections were most troublesome traits that pose a great challenge to therapy due to its low antibiotic susceptibility and the antagonistic effect on other bacteria particularly normal intestinal flora may support this organism to establish themselves inside the human system really a threat to the clinical settings.

Moreover haphazard use of antibiotics may also lead to produce more MDR strains and to avoid this a more restricted and rational use of antibiotics should be prescribed with regular monitoring of virulence characters and encoding genes both at clinical and molecular level to provide better treatment. Therefore distinguishing the trends in resistance of *P.aeruginosa* becomes important for choosing the right antibiotic. Our study will be an eye opener for those handling clinical samples to be aware of this organism and also guide for effective control measures to combat the organism.

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