IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 20, Issue 1 Ser.1 (January. 2021), PP 42-46
www.iosrjournals.org

Study of detection of *plcH gene* and its phenotypic expression in *Pseudomonas aeruginosa* isolated from various clinical samples.

J.D.Andhale[1], R.N Misra[2]
[1] *Associate Professor, Department of Microbiology, Ananta Institute of Medical Sciences, Hospital and Research Center,Rajsamand,Rajasthan,India-313202.*
[2] *Professor and Head, Department of Microbiology, Dr.D.Y.Patil Medical College, Hospital and Research Center (Dr.D.Y.Patil Vidyapeeth) Pune, India-411018*

Abstract:
Background: *Pseudomonas aeruginosa (P.aeruginosa) is an opportunistic pathogen and can infect almost all tissues. After multiplication and colonization, P.aeruginosa spreads within cells. Toxins and enzymes produced by P.aeruginosa are key factors that invade host cells and produce disease. P. aeruginosa harbours virulence genes like plcH, plcN, plcB, exoS, exoT, exoU, lasB, pilB, exoS, algD, nanI, pvdA. These virulent genes colonize the host cells and play important role in causation of disease. Phospholipases C involve in pathogenicity. P.aeruginosa produces two types of phospholipases C, haemolytic phospholipase C and non-haemolytic* phospholipase *C encoded by plcH and plcN genes respectively. Haemolytic phospholipase C is responsible for haemolysin production and show haemolytic activity. Phospholipases C are secreted via a micro machine known as Type -2 secretion system. Phosphate deficiency induces production of haemolytic phospholipase C. This study was focussed on study of detection of plcH gene and its and phenotypic expression in P.aeruginosa strains isolated from various clinical samples obtained at Tertiary Care Hospital.*
Objectives: *Detection of plcH gene and its phenotypic expression in P. aeruginosa isolated from various clinical samples received in a Tertiary Care Hospital.*
Materials and methods: *Thirty strains of P. aeruginosa isolated from clinical specimens were identified using standard laboratory methods. Gene plcH were detected by polymerase chain reactions and gel electrophoresis technique. Production of haemolysins was studied on 5% sheep blood agar plates.*
Results: *PCR amplification results showed presence of plcH genes in 22 (73.33%) out of 30 P.aeruginosa strains and 21 (70%) of the isolates showed haemolysin production.*
Conclusion*: Gene plcH of p. aeruginosa is one of the important virulent factors and plays key role in development of disease. It is concluded that plcH gene can be a striking pathogenic factor shown by presence of 73.33% and expressed phenotypically by 70% of P. aeruginosa strains isolated from clinical samples. The proved role of plcH virulent gene in the causation of disease would help in geeting clue of the prognosis of infections caused by Pseudomonas and scheming successful therapy and designing suitable vaccine against the prevention of infections caused by Pseudomonas.*
Key words: *P.aeruginosa, plcH, Haemolysin, PCR.*

Date of Submission: 20-12-2020 Date of Acceptance: 03-01-2021

I. Introduction

P. aeruginosa is Gram negative, motile, rod shaped opportunistic pathogen. It causes chronic and acute infections in human. Infections caused by *P. aeruginosa* play major role in cystic fibrosis, sepsis and in burn patients. Infections caused by *P. aeruginosa* are commonly observed in immune suppressed patients. [1-5].

A serious issue in infections caused by *P.aeruginosa* is multi-drug resistance to routinely used antibiotics to treat patients [6-7]. These high and multi-drug resistance is acquired due to genetic, intrinsic and acquired resistance [8]. *P.aeruginosa* is more virulent as it produces large number of cellular and extracellular virulence factors regulated by quorum sensing system. These virulence factors contribute in causation of disease in humans [9].

Virulence factors produced by *P.aeruginosa* play an important role in pathogenicity are exotoxins and exoenzyme U, exoenzyme S, exoenzyme T secreted by T3 secretion system encoded by *exoU, exoS* and *exoT* virulent genes respectively and elastage B encoded by *lasB. P.aeruginosa* produces haemolytic phospholipase C and non-haemolytic phospholipase C which are encoded by *plcH* and *plcN* virulence genes respectively. Phospholipid present in surfactans may be hydrolysed by phospholipases C [10-11].In India, very few researchers have worked on virulence factors like haemolytic phospholipase C and its allied *plcH* gene produced by *P. aeruginosa*. Considering these facts in mind, this research study was designed to detect and study the

distribution of *plcH* virulence gene and its phenotypic expression in *P. aeruginosa* isolated from various clinical samples obtained at Tertiary Care Hospital.

Aims and objectives: Aims and objectives were detection of *plcH* gene and its phenotypic expression in *P. aeruginosa* isolated from various clinical samples received in a Tertiary Care Hospital.

II. Materials And Methods:

This study was carried out at the Department of Microbiology, in Dr.D.Y.Patil Medical College, Hospital and Research Cenre, Pune-411018.

Various clinical samples like urine, sputum, pus, blood and body fluids, were received from different clinical wards including of all ages and both sexes for routine culture and sensitivity tests. All different clinical samples received were processed and confirmed strains of *P.aeruginosa* were screened for detection of *plcH* gene by Polymerase chain reactions technique and production of haemolysins was studied on 5% sheep blood agar plates.

Ethical statement

This research study was approved by Institutional Ethical Committee of Dr.D.Y.Patil Medical College, Hospital and Research Centre (Dr.D.Y.Patil Vidyapeeth), Pune.

Source of Funding: None.

Coflict of Interest: None.

Isolation and Identification of *P.aeruginosa*

All different clinical samples received were inoculated onto nutrient agar, blood agar and MacConkey agar plates. After inoculation and incubation 37ºC for 24 hrs, plates were examined for presence of growth. *P.aeruginosa* was confirmed by studying colony morphology, pyocyanin pigments production, typical grape like odour, tendency to growth at 42ºC, Gram staining, motility test, positive citrate and oxidase tests [12].

Detection of haemolysin production of *P.aeruginosa* isolated from various clinical samples

Detection of Hemolysins: All 30 strains of *P.* aeruginosa isolated from various clinical samples under this study were screened for detection of haemolysin production activity. *P.aeruginosa* grown for 18 hours at 37ºC in nutrient broth were plated on blood agar containing 5% (vol/vol) sheep blood to obtain isolated colonies. Plates were incubated at 37ºC for 24 hours. The clear zone around the colonies (total lysis of red blood cells around the colonies) was considered as positive reaction for production of haemolysins [13, 14].

Detection of *plcH* gene of *P.aeruginosa* isolated from various clinical samples

Extraction of DNA:

For the screening of *plcH* virulence genes of *P.aeruginisa,* Chromosomal DNA from the 30 clinical strains of *P.aeruginosa* clinical isolates under this study was extracted. Purification of extracted DNA was carried out using (Ganeaid-Presto™ Mini gDNA bacteria Kit) a commercial available DNA extraction kit following the manufacturer's guidelines.

Polymerase Chain Reaction: The sequences of the primers used in polymerase chain reactions for detection of *plcH* gene and its molecular weight are shown in Table No.1.

Table No.1: The Primer sequence used for the screening of *plcH* genes.

Gene	Primer sequence	Amplicon size	Length(bp)	References
plcH	Forward 5'-GAAGCCATGGGCTACTTCAA-3' Reverse 5'-AGAGTGACGAGGAGCGGTAG-3'	20 20	307	15, 16

The chromosomal DNAs extracted from *P.aeruginosa* strains under study were used as templates for polymerase chain reactions. Polymerase chain reactions were carried out in 25ul mixture containing 7.5ul distilled water, 1.5ul forward primers, 1.5ul reverse primers, 2.0ul DNA template and 12.5ul mastermix (Geneaid-Presto™Mini gDNA bacteria Kit).

The Polymerase chain reactions were carried out using conditions as shown in Table No.2

Table 2: Conditions used for Polymerase Chain Reactions.

Gene	Initial denaturation	No.of Cycles	Denaturation in each cycle	Annealing	Primer extention	Final extention
plcH	95ºC, 2 min	30	95ºC, 30 sec	55ºC,30 sec	72ºC, 30 sec	72ºC,5 min

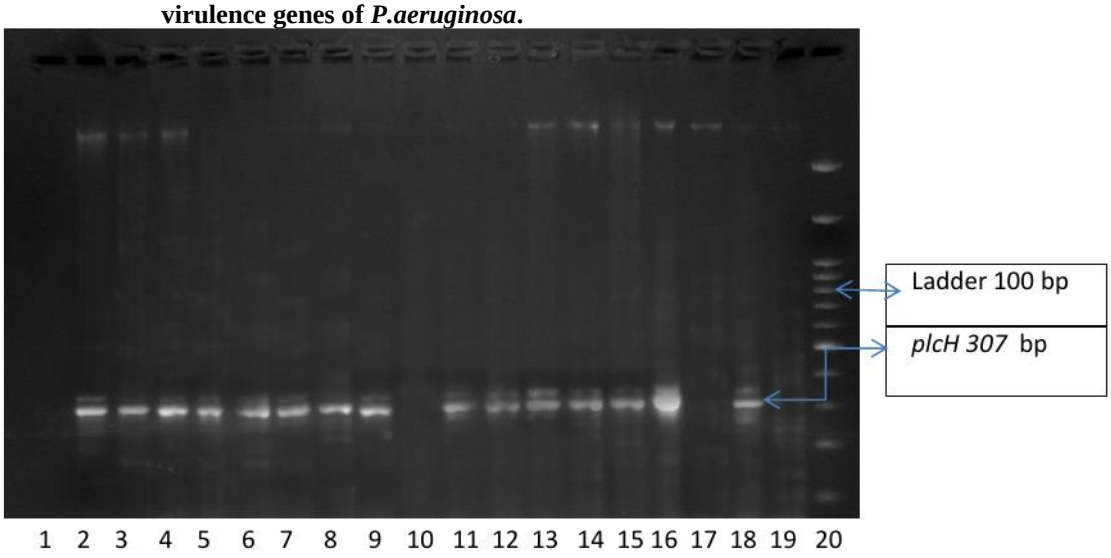
Gel electrophoresis

Polemerase chain reaction products of *P.aeruginosa* were used for gel electrophoresis. For a gel preparation, 250 ml agarose quantity was required. Agarose gel was prepared with 2% agarose and ethidium bromide as it shows good resolution for small fragments. Images of PCR products were detected using transillumator by UV illumination as shown in image No.1. For the estimation and to compare size of PCR products, the 100bp DNA molecular size markers were used [17].

III. Results:

This research study was carried out using 30 strains of *P.aeruginosa* isolated from various clinical samples in respect to sites of infections as shown in Table No.3

Image No.1: Images of Gel Electrophoresis and amplification Products of *plcH* virulence genes of *P.aeruginosa*.



No.1= Negative control (Distilled water),Nos,2,3,4,5,6,7,8,9,11,12,13,14,15,16,18 showing *plcH* gene of *Pseudomonas aeruginosa*. .Nos 10, 17, &19= showing no *plcH* gene. & No.20 is ladder100 bp

Table No.3 Distribution of *plcH* genes of 30 *P.aeruginosa* clinical Isolates in respect to sites of Infections.

S.N	Sample	case	*plcH*		S.N	sample	Case	*plcH*
1	Pus	Maxilla	+		**16**	Urine	UTI	-
2	Pus	CSOM	+		**17**	pus	CSOM	+
3	Pus	NHTU	+		**18**	pus	CSOM	-
4	Pus	Hydrocele	+		**19**	urine	UTI	+
5	Urine	CUTI	+		**20**	blood	Fever	+
6	Urine	UTI	+		**21**	pus	Leg abscesses	-
7	Pus	Leg Cellulitis	+		**22**	pus	NF	+
8	Pus	DFU	+		**23**	pus	NF	+
9	Fluid	COPD	-		**24**	sputum	RTI	+
10	Pus	NF	+		**25**	pus	TA	-
11	Pus	DFU	+		**26**	pus	DFU	-
12	Pus	CSOM	+		**27**	pus	DFU	-
13	Urine	UTI	+		**28**	sputum	RTI	+
14	Sputum	RTI	+		**29**	blood	Pneumonia	+
15	Sputum	RTI	+		**30**	pus	DFU	-

CSOM = chronic suppurative otitis media, NHTU=Non Healing Tropic Ulcer, UTI=Urinary Tract Infection, NF=Necrotizing fascitis, COPD=Chronic Obstructive Pulmonary Disease, DFU=Diabetes Foot Ulcer, RTI=Respiratory Tract infection, TA=Traumatic Amputation, CAUTI =Catheter Associated UTI.

Out of 30 strains of *P.aeruginosa* clinical isolates, 22(73.33%) showed amplification of *plcH* gene and haemolysin production was seen in 21 (70%) as shown in Table No.4,

Table No.4 Showing specimen-wise distribution of PlcH gene and its phenotypic expression (haemolysin production).

S.N	Source	*plcH* gene (%)	Hemolysis (%)
1	Pus	12(66.66)	11(61.11)
2	Urine	04 (80)	04(80)
3	Sputum	04(100)	04(100)
4	Blood	02(100)	02(100)
5	Fluids	00(00)	0(0)
6	Total	22(73.33)	21(70)

IV. Discussion

The aims and objectives of this research were to detect *plcH* virulence gene and its phenotypic expression in *P.aeruginosa* isolated from different clinical samples. In this research study, 30 strains of *P.aeruginosa* clinical isolates were included.

P.aeruginosa possesses number of quarum sensing genes which encode for number of exotoxins and exoenzymes. These exotoxins and exoenzymes are virulence factors playing their active roles in pathogenicity of various infections caused by the organism.

There are total 23 so far known bacterial virulence genes, namely *lasl, lasB, lasA, lasR, rhlR, rhIl, rhlAB, fliC, aprA, plcN, plcH, toxA, ExoT, ExoS, ExoY, ExoU, phzI, phzll, phzS, phzM, pilA, pilB* and *pvdA* [18].

The present paper discuss only the *plcH gene* encoding for haemolysin proteins. *plcH* is an important virulence genes encoding for haemolytic phospholipase C. Hemolytic phospholipase C play an important role in spreading type of infections caused by *P.aeruginosa*. Finding out its prevalence is important, as an epidemiological marker in pathogenic *P.aeruginosa.*

In this study, out of 30, 22 (73.33%) *P.aeruginosa* clinical strains were positive for *plcH* gene. This finding is in agreement with similar studies where the prevalence of *plcH* was documented as 70.42% and 75% respectively [19, 20]. Other researchers have reported high incidence of *plcH* gene in *P.aeruginosa* strains isolated from different clinical specimen in hospitals [21, 22, 23]. In pus samples, 66.66% isolates showed presence of *plcH* gene and 80% *P.aeruginosa* strains obtained from urine samples were positive for *plcH* gene. All *P.aeruginosa* strains were positive for *plcH* gene isolated from blood and sputum. Therefore, detection of *plcH* gene can be conveniently used as one of the important markers of virulence in epidemiological study.

The presence of *plcH* and its encoded product determine the prognosis of infections caused by *P. aeruginosa* and also predict the possibility of development of spreading type of infections. In the present study, 70% (21/30) of *P.aeruginosa* clinical isolates showed haemolysin production.Haemolysin production was found highest (100%) in blood and sputum isolates showing expression of *plcH* gene and suggesting that the haemolysin virulence factor is highest in spreading and systemic type of infections caused by *P. aeruginosa*. In other research studies showed that in majority of infections caused by *P.aeruginosa*, the *plcH* and its phenotypic expression were reported at 100 proportion [24, 25]. Such spreading type infections caused by *plcH gene* possessing strains of the organism had high morbidity and were more difficult to treat. The multi-drug resistance in such strains further aggravate infections resulting in high morbidity and mortality. Researchers, therefore suggest that the *Pseudomonas aeruginosa* clinical isolates having *plcH* gene, must always to subject to drug susceptibility testing in order to manage the infections effectively.

Statistical analysis:

By using Fisher exact test, the 'P' value is 0.00001. As the $P < 0.05$, result is significant and hence, there is strong association between *plcH* genes and its phenotypic expression.

Therefore either the detection of presence of *plcH* gene or its phenotypic expression can be used to determine the development and prognosis of spreading type of infections caused by *P. aeruginosa.*

V. Conclusion

We found that majority of the *P.aeruginosa* strains isolated from patients having spreading type of infections possessed *plcH* gene and its corresponding phenotypes.

We also found that since there is strong correlation between the presence of *plcH* gene and its phenotypic expression ($P<0.05$); either of the in-vitro detection of *plcH* gene or its phenotypic expression can be used to determine the severity and prognosis of pseudomonas infections in patients.

The *plcH* gene detection by polymerase chain reaction is rapid method than the detection of its phenotypic expression and therefore would be more helpful in epidemiological study and in deciding the treatment course for the infections caused by *P. aeruginosa.*

References:

[1]. Doring G. Chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. In:Campa M, Bendinelli M, Friedman H (eds) *Pseudomonas aeruginosa* as an opportunistic pathogen.New York, NY, Plenum Press. 1993: 245-73.

[2]. Jacome PR, Alves LR, Cabral AB, Lopes AC, Maciel MA. Phenotypic and molecula characterization of antimicrobial resistance and virulence factors in Pseudomonas aeruginosa clinical isolates from Recife, State of Pernambuco, Brazil. Rev Soc Bras Med Trop 2012; 45:707-12.

[3]. Bergmann U, Scheffer J, Koller M *et al.* Induction of inflammatory mediators (histamine and leukotrienes) from rat peritoneal mast cells and human granulocytes by *Pseudomonas aeruginosa* strains from burn patients. *Infect Immun* 1989: 57: 2187-95

[4]. Aious V, Navon-Veneziz S, Seigman-Igra Y,Camel Y. Multi-drug resistant Pseudomonas aeruginosa: Risk factors and clinical impact.Antimicrob Agents Chemother.2006; 50(1):43-8

[5]. Baltimore RS, Christie CD, Smith GJ. Immunohistopathologic localization of *Pseudomonas aeruginosa* in lungs from patients with cystic fibrosis. Implications for the pathogenesis of progressive lung deterioration. *Am Rev Respir Dis* 1989; 140:1650-61.

[6]. Preeti BM, Manjanath PS. Antimicrobial susceptibility pattern of *aeruginosa* from clinical isolates at atertiary care centre of in Jivaypur Karna.Journal of Chemical and Pharmaceutical Research. 2015; 7(8):186-90.

[7]. Fluit AC, Verhoef J, Schmitz FJ, The European sentry participants. Antimicrobial resistance in European isolates of *Pseudomonas aeruginosa.* Microbiol Dis. 2000; 19:370-74.

[8]. Hancock REW, Spreet DP. Antibiotic resistance in *Pseudomonas aeruginosa* Mechanisms and impact on treatment. Drug Resistance Updates. 2000; 3:247-55.

[9]. Van-Delden C, Iglewski BH. Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerg Infect Dis. 1998; 4:551–60.

[10]. Yahr TL, Hovey AK, Kulich SM, Frank DW.Transcriptional analysis of the *Pseudomonas aeruginosa* exoenzyme S structural gene. J Bacteriol. 1995; 177:1169 – 78.

[11]. Jaffar-Bandjee MC, Lazdunski A, Bally M,Carrere J, Chazalette JP, Galabert C.Production of elastase, exotoxin A, and alkaline protease in sputa during pulmonary exacerbation of cystic fibrosis in patients chronically infected by *Pseudomonas aeruginosa.* J Clin Microbiol. 1995; 33:924–29.

[12]. Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A. 1996. Mackie and McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone.

[13]. Craig W, Kaye SB, Timothy JN, Chilton HJ, Miksch S, Hart AC. Genotypic and phenotypic characteristics of P. aeruginosa isolates associated with ulcerative keratitis. J Med Microbiol. 2005; 54:519, 460, 526.

[14]. Cotar AI, Chifiriuc MC, Dinu S, et al. Screening of molecular Virulence of markers in Staphyloccus aureus and Pseudomonas aeruginosa strains isolated from clinical infections. IntJ Mol Sci. 2010; 11:5273–91.

[15]. Cotar AI, Chifiriuc MC, Banu O, Lazer V. Molecular Characterization of Virulence Patterns in Pseudomonas aeruginosa strains isolated from respiratory tract and wound sample. Int J Mol Epidemiol Genet.2014; 3:125–34.

[16]. Panagea S, Winstanley C, Parsons YN, Walshaw MJ, Ledson MJ, Hart CA. PCR based detection of cystic fibrosis epidemic strain of Pseudomonas aeruginosa. Mol Diagn. 2003; 7:195–200.

[17]. Fritsch EFTM, Sambrook J. Molecular cloning-a laboratory manual, 7th edition, Cold Spring Harbor Laboratory, Principles of Gene Manipulation.

[18]. Ertugrul BM, Erman Oryasin, Lipsky BA, Willke A, Bozdogan B. Virulence genes fliC, toxA and phzS are common among Pseudomonas aeruginosa isolates from diabetic foot infections. Infectious Disease. 2017; 50 (4):273–9

[19]. Heidary Z, Bandani E, Eftekhary M, Jafari AA. Virulence Genes Profile of Multidrug Resistant Pseudomonas aeruginosa Isolated from Iranian Children with UTIs.Acta Med Iran.2016; 54(3):201-10

[20]. 165 Sabharwal N, Dhall S, Chibber S, Harjai K. Molecular detection of virulence genes as markers isolated from urinary tract infections. Int J Mol Epidemiol.2014; 5:125-34.

[21]. Wolska K, Szweda P. Genetic Features of clinical Pseudomonas aeruginosaStrains. Pol J Microbiol. 2009; 5:255–60.

[22]. Mitov I, Strateva T, Markova B. Prevalence of Virulence Genes AmongBulgarian Nosocomial and Cystic Fibrosis Isolates of Pseudomonas aeruginosa. Braz J Microbiol. 2010; p. 588–95.

[23]. Espinosa MR, Gloria SC, Gabriela DS, Luisa SM, J L Mendez .Genetic and Phenotypic Characterization Of Pseudomonas aeruginosa Population With High Frequency Of Genomic Islands. Plos one 2012; 7:1–11.

[24]. Lanotte P, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A,et al. Genetic Features Of Pseudomonas aeruginosa Isolates From CysticFibrosis Patients Compared With Those Of Isolates From Other Origins.J Med Microbiol. 2004; 53:73–81.

[25]. Antonov VA, Altukhova VV, Savchenko SS, Tkachenko GA, Zamaraev VS, et al. Molecular genetic analysis of Pseudomonas aeruginosa strains isolated from environment and patients in health care facilities.Zh Microbiol Epidemiol Immunobiol.2010;8-13.

J.D.Andhale, et. al. "Study of detection of plcH gene and its phenotypic expression in Pseudomonas aeruginosa isolated from various clinical samples." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 20(01), 2021, pp. 42-46.