Research Article

Genotyping Diversity of *Proteus vulgaris* by using RAPD-PCR and Study of Some Factors on the Activity of Bacteria

Lamees A. Abdul-Lateef

Lamees A. Abdul – Lateef
Department of Microbiology, College of Medicine, University of Babylon, Iraq

Correspondence:
Lamees A. Abdul – Lateef
Department of Microbiology, College of Medicine, University of Babylon, Iraq
E-mail: dr.lamees.razzak@gmail.com

ABSTRACT

P. vulgaris causes variety of infection such as, UTIs, wound infections, burn infections, bloodstream infections, and respiratory tract infections, bacteremia and brain abscesses. A total 120 specimen, were collected from clinical samples (urine, vagina, wound, and burn). only 18 isolates of Proteus vulgaris were recovered. The relationship between 18 Proteus vulgaris isolated using RAPD-PCR was performed. The ten primer showed polymorphism among the isolates generating 86 bands, 54 of which were polymorphic with sizes ranging between 150-30000bp. The mean percentage of ten primer 62.79%. Proteus vulgaris isolates identified were screened for biofilm production by TCP method. Among the isolates, 55.55% were biofilm producer and 44.44% were non/weak biofilm producer. Regard the effect of NAC (10%) on biofilm formation was also studied, this study proved that NAC effectively reduced the viability of biofilm formation from (55.55) to (5.55%). On the other hand, the effect of NAC and green tea at concentration (0, 1, 5, 10, and 15 mg/mL) on urease was also investigated, it was state the high concentration of it could cause inhibition to urease production.

Keywords: *P. vulgaris,* cytoplasm, RADP typing.

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INTRODUCTION

Proteus vulgaris is gram-negative bacteria found as a part of normal flora of human gut and also widespread in the environment. *Proteus* was ranked as the third cause of hospital-acquired infections [1]. It is rod, non-spore former, motile and chemo-heterotroph organism, with a wide range variable transmission mode [2]. *P. vulgaris* has been reported to cause wound infections, UTIs, bloodstream infections, burn infections, and respiratory tract infections [3][4]

Proteus has an increasing clinical significance [5], this enforced the need for picking of an efficient method, molecular fingerprinting is of countless significance epidemiologically. Bacterial-Genotyping opened large number of chances for variable epidemiological works, aiding in the diagnosis and identification of environmental and clinical isolates, monitoring of clone dissemination, evaluation of their relationships, and categorization of different bacterial population groups within less or more restricted environments [6]. RAPD, a method based on PCR process, is used in the detection and identification of clinical isolates of *Proteus mirabilis* [7] also *Proteus penneri* [8]. The genetic relationship among the closely related bacterial population can be analysed by a good tool; the usage of genetic analysis using RAPD typing.

Urease is an enzyme that considered as one of the centrally important virulence factors [9]. It contains nickel as an active core and generates ammonia from destruction of urea, that increasing urine alkalinity which favours the formation of carbonate apatite crystals and struvite. Additionally, it aids in the chemotactic activity of bacteria, with lessening bacterial opsonization by complement

components of human [10][11][12]. Urease enzyme of *Proteus* spp. differs from that of other bacterial types, as it is present not only in the cell cytoplasm but also present in the bacterial surface [13][14].

However, Biofilms are microbial communities consisting of bacteria, capable to self-reproduce on biological surfaces, causing infections. These biofilms are resistant to antibacterial treatments and host immune response. Biofilm is a sort of structurally complex ecosystem that allows bacteria to survive to inhospitable conditions, becoming responsible of infections in different tissues and organs [15]

The N-Acetyl-cysteine (NAC) had been applied to control bacterial biofilm formation and growth, as an alternative pharmacological method in human diseases. Several studies were informed that NAC reduces biofilm formation among different bacterial spp [16][17][18]. Moreover, NAC can shrink the ability to produce extracellular polysaccharide matrix [19] and enhancing interruption of mature biofilm [20].

The use of plant extracts and their products has been valuably increasing around the world, in order to abate side effects of chemical drugs [21]. Green tea can be obtained from Camellia sinensis (Theaceae family) is a beverage that widely utilized by people to achieve a dietary source of biologically active complexes; which carry large benefits to human health. Extracts of Green tea contain caffeine, tannin, catechins and polyphenols, these extracts are widely applied to study and verify the pharmacological activities of green tea. It had been demonstrated significant anti-carcinogenic, probiotic, antioxidant, antimicrobial and anti-inflammatory [22][23].

MATERIAL AND METHODS

Patients

Out of 120 samples, only eighteen isolates of *Proteus vulgaris* were obtained from different samples includes (urine, vagina, wound and burn) from patients by standard bacteriological methods. All samples were obtained from persons, who admitted to Al-Hilla General Teaching Hospital and Merjan Medical City in Babylon Governorate during a period of three months lasting from (August 2018 to October 2018).

Bacterial identification

The samples has been inoculated on culture media and aerobically incubated at 37°C for 24hr. Then the bacterial isolates was performed by using automated VITIK-2 compact by using VITEK®2 GN kit.

DNA extraction from gram-negative bacteria

DNA extraction was done according to the genomic DNA purification kit (Geneaid, UK)

Genotyping detection by using RAPD-PCR

The primers and PCR condition used to amplified genotyping of *Proteus vulgaris* at table (1). PCR reaction 25 µl of contained 2.5 µl RAPD primer, 5 µl DNA, 12.5 µl master mix and 5 µl free nuclease water. The product of PCR was visualized by electrophoresis on 2% agarose

Table 1: Primers of genotyping of *Proteus vulgaris* by RAPD-PCR

Gene	Primer sequence	Size (bp)	PCR condition	Reference
OPA10	5' GTGATCGCAG 3'	150-3000		
OPA11	5' CAATCGCCGT 3'			
OPD20	5' ACCCGGTCAC 3'		92°C 4 min 1x	[24]
OPX13	5' ACGGGAGCAA 3'		92°C 1 min	
OPX15	5' CAGACAAGCC 3'		40°C 1 min 40x	
OPZ04	5' AGGCTGTGCT 3'		72°C 5 min	
OPZ08	5' GGGTGGGTAA 3'		72°C 5 min 1x	
OPZ10	5' CCGACAAACC 3'			
OPZ19	5' GTGCGAGCAA 3'			
OPZ20	5' ACTTTGGCGG 3'			

Effect of N-acetyl-L-cysteine on biofilm formation
Biofilm formation was done by tissue plate method described by [25] was considered as standard test for detection of biofilm and classified as described in table (2)

Table 2: Classification of bacterial adherence by TCP method

Mean OD values	Adherence	Biofilm formation
< 0.120	Non	None / Weak
0.120 – 0.240	Moderately	Moderate
> 0.240	Strong	High

However, the same procedure was described in tissue culture method plate was done with modification. N-acetyl-L-cysteine (10%) was added to samples in wells of sterile polystyrene 96 well-flat bottom tissue culture plate, after fixed with sodium acetate for half an hour and all steps done as the same steps that described previously [26].

Effect of N-acetyl-L-cysteine and green tea on urease assay

Overnight culture of *P. vulgaris* was diluted 1:1000 in LB containing ampicillin (50 mg/mL) and at 0, 1, 5, 10, and 15 mg/mL and was incubated for 3 h. The bacteria were then induced with urea (0-500mg/ml) and harvested after 3 hr. of additional growth. Induced bacteria were washed twice in phosphate-buffered saline (8g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 per litre (pH 7.4)), the absorbance was read at wave length OD600 [27].

Water extract of green water were prepared by applying 50 gm of commercially used dry green tea in 100 ml of boiled water, then it allowed to cool in order to be used in above procedure to detect its effect on urease activity.

RESULTS AND DISCUSSION

Detection of genotyping diversity of *Proteus vulgaris* isolates from different clinical samples by RAPD-PCR

*Molecular diversity of *Proteus vulgaris* isolates by RAPD – PCR:

The total of 86 DNA amplified fragments with the ranging of size from 150-3000bp were detected by using ten random primers (OPA-10, OPA-11, OPD-20, OPX-13, OPX-15, OPZ-04, OPZ-08, OPZ-10, OPZ-19, OPZ-20), whereas 54 fragments were polymorphic and other 32 fragments were ideated between the 18 *Proteus vulgaris* isolates as shown in table (3).

The ten primer showed a mean polymorphism of 62.79% where percentage of primes OPA-11 was higher (75%), however primer OPZ-04 display lower percentage with 14.28%.

Table 3: primers reveled the genetic discriminations between *Proteus vulgaris* isolates by using PCR -RAPD

S/N	Operon code	No. of amplified fragments	No. of polymorphic bands
1	OPA10	5	5
2	OPA11	12	9
3	OPD20	3	2
4	OPX13	13	9
5	OPX15	8	3
6	OPZ04	7	1
7	OPZ08	10	8
8	OPZ10	7	5
9	OPZ19	14	7
10	OPZ20	7	5
	TOTAL	86	54

Primer (OPA10) revealed 5 fragments, five fragments were polymorphic with ranging size from 250 to 1500 bp as shown in fig (1). The number of fragments of bacterial isolates was variable in their fragments in a decrease from 5 to 1. Two isolates (16, 11) were seen the highest with 5 fragments, followed by isolate 1 with 4 fragments while isolate number 12 revealed two fragments. While the isolate number (7) was shown one fragment.

Figure 1: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPA-10* RAPD primer. Lane M: ladder 100-3000. 1, 7, 11, 12, 16 were positive for *OPA-10* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPA-11) display 12 fragments, nine of polymorphic with size ranging from 270 to 2500bp as shown in fig. (2). The no. of fragments of the isolates were divers. The isolate no. (1) has 9 fragments, however, the no. of fragments were decreased until reach six fragments in isolate no. (15) and five fragments in isolate no.(8)

Figure 2: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPA-10* primer. Lane M: ladder 100-3000. Only 1, 8, 15 were positive for *OPA-10* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPD-20) revealed 3 fragments, 2 fragments of polymorphic with size ranging from 150 to 1800bp as shown in fig (3). The number of fragments of isolates were variable in their fragments in decreasing in fragment from 2 to 1.

Five isolates no. (2, 5, 8, 9, and 14) have two fragments. However, the isolates (2 and 5) and isolates (8 and 9) with similar fragment size. While the isolates no. (18) has only one fragment.
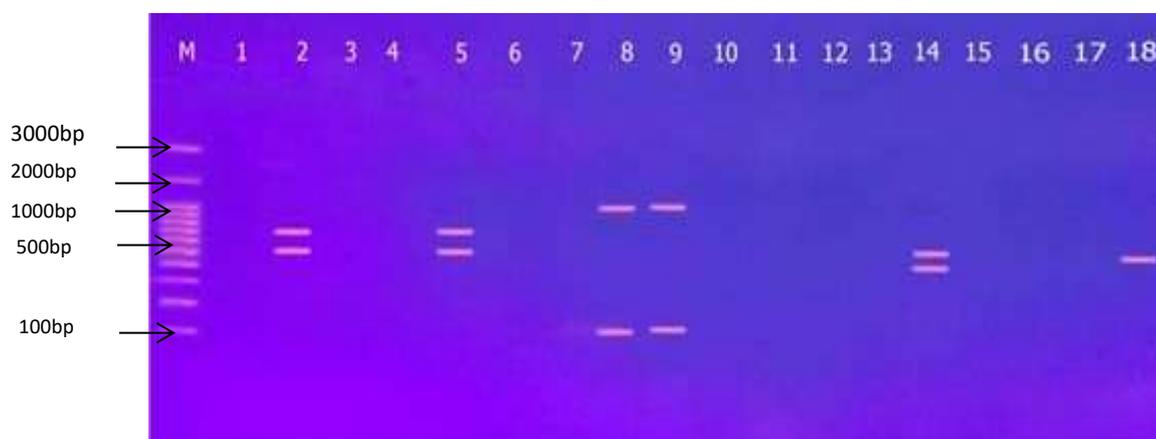

Figure 3: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPD-20* primer. Lane M: ladder 100-3000. 2, 5, 8, 9, 14, 18 were positive for *opw-10* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPX-13) revealed 13 fragments, nine fragments of polymorphic with size ranging from 250 to 3000 bp as shown in fig. (4). Two isolates no. (12 and 13) show the high no. of fragment reach to 9 fragments, followed by isolate (7)

with 3 fragments, while isolate (8) with two fragment and isolate (15) with 1 fragment. However, 12 and 13 isolates were similar in fragment sizes.

Figure 4: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPX-13* primer. Lane M: ladder 100-3000. 2, 7, 8, 12, 13, 15 were positive for *OPX-13* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPX-15) revealed 8 fragments, 3 fragment of polymorphic with ranging of size from 150 to 1800 as shown in fig (5). The no. of fragments were descending from 3 to 1.

four isolates (2, 5, 8 and 14) show the 3 fragments, followed by isolate 9 with two fragments while isolate 18 revealed one fragment.
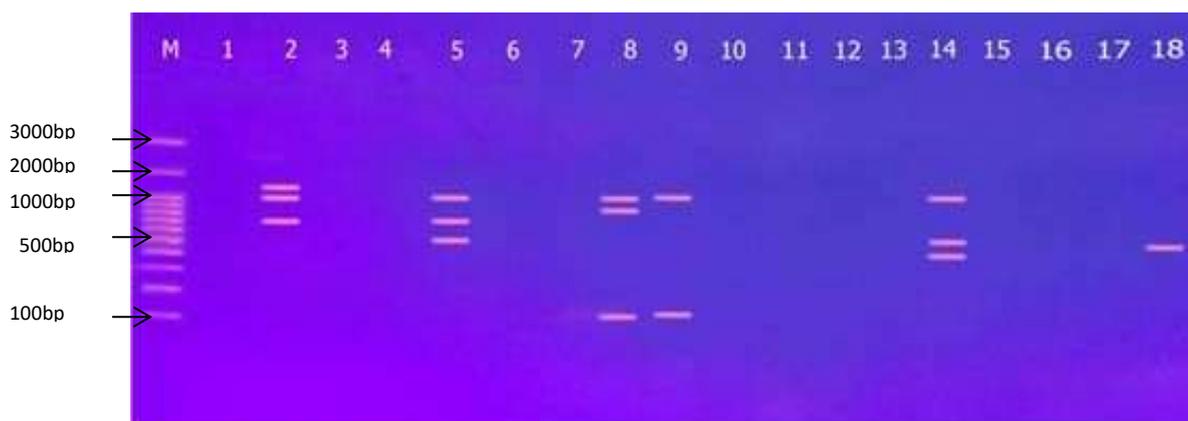
Figure 5: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPX-15* primer. Lane M: ladder 100-3000. 2, 5, 8, 9, 14, 18: were positive for *OPX-15* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPZ-04*)* revealed 7 fragments, 1 fragment of polymorphic with ranging of size from 300 to 900 bp as shown in fig (6). The fragment size varied between isolates. However the four isolates (2, 3, 9, and 12) revealed only one fragment

Figure 6: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPZ-04* primer. Lane M: ladder 100-3000. 2, 3, 9, 12 were positive for *OPZ-04* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPZ-08) revealed 10 fragments, 8 fragment of polymorphic with ranging of size from 200 to 3000 bp as shown in fig. (7). The number of fragments of isolates were variable. The no. of fragments in isolates was descending from 8 to 1. One isolate number (17) show high no. of fragment reach to 8 fragments, the isolates (1,16 and 18) has 6 fragments while isolates (3,7) show five fragments. The others isolates reveled descending in no. of fragment until reach 4 fragments in isolates (2,4,6 and 11) .while 3 fragment in isolate 15 and one fragment in isolates (8 and 13).

Figure 7: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPX-08* primer. Lane M: ladder 100-3000. 1-4, 6-8, 11, 13, 15-18 were positive for *OPX-08* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPZ-10) revealed 7 fragments, five fragments of polymorphic with ranging of size from 200 to 2800 bp as shown in Fig (8).Two isolates (15 and 18) show the high no. of fragment reach to five fragments, while the isolates no. (7, 8, and 10) has four fragments, the number of fragments decreased until reach to one fragment in isolates (1, 6, and 17).
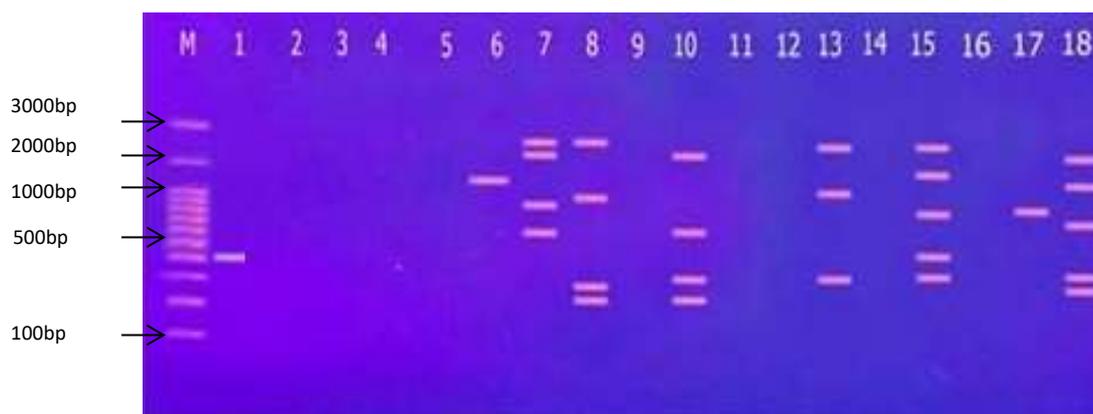

Figure 8: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPZ-10* RAPD. Lane M: ladder 100-3000. 1, 6-8, 10, 13, 15, 17, 18 were positive for *OPZ-10 gene.* 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPZ-19) revealed 14 fragments, 7 fragments of polymorphic with range of sizes from 200 to 2700 bp as shown in Fig (9). The numbers of fragments of the isolates various greatly. Two isolates 17 and 18 showed the high fragments with (7), followed by isolates 16 with 6 fragments while isolates (1, 3) with 4 fragments. Also isolates (5, 6, 12, 13) with 3 fragments .The remaining isolates (4, 9, and 15) contained only one fragment.
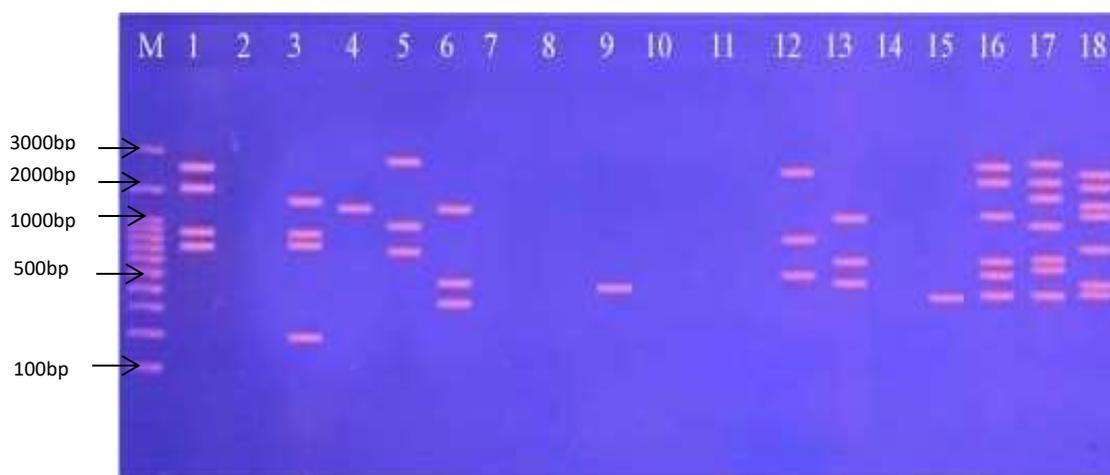
Figure 9: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPZ-19* primer. Lane M: ladder 100-3000. 1, 3-7, 12, 13, 14, 15-18. Positive for *OPZ-19* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

Primer (OPZ-20) revealed 7 fragments, five fragments of polymorphic with ranging of sizes from 200 to 2000 bp as shown in fig (10). The no. of fragments in isolates was decreased from 5 to 3. Two isolates (16 and 17) show the high no. of fragments reach to five fragments, while isolate (6) with four fragment, and isolate (2) with three fragments.

Figure10: RAPD fingerprinting of 18 *P. vulgaris* isolates obtained by *OPZ-20* primer. Lane M: ladder 100-3000. 2, 6, 16, 17. Positive for *OPZ-20* gene. 1-4: no. of isolates from wound, 5-8: no. of isolates from burn, 9-11: no. of isolates from vagina, 12-18: no. of isolates from urine

*Genetic similarity of *P. vulgaris* isolates by RAPD analysis:
The genetic similarity between isolates of *P. vulgaris* was done using PAST 3 as shown in fig (11).
Based on the RAPD-PCR dendrogram, there well distinguishable fingerprint pattern in all isolates. All *P. vulgaris* isolates except (Pv 9 vagina) were related at least (8%) similarity. The dendrogram analysis showed that the *P. vulgaris* isolates distributed into two clusters
The first cluster include one isolate (Pv 9 vagina) while the second cluster include 17 isolates. However, second cluster divide the (17) bacterial isolates into 2 sub-cluster, while the first sub cluster contain (15) isolates one of them contain isolate (Pv 13 urine) and the second sub-cluster contain (14) isolates and sub sequent divided the sub-cluster into two branches. The first branch contained 6 isolates, these branch contained two sub branches, one contained one isolate (Pv 8 burn). While, the second was more divided into two sub-branches. The first sub-branch contained isolates (Pv 10 vagina) and (Pv 12 urine) with similarity (43%) and the

second sub-branch contained three isolates one isolate (Pv 15 urine) and the two isolates (Pv 2 wound) and (Pv 3 wound) with similarity (40%).
Besides, the second branch contain (8) bacterial isolates and divided into 2 sub-branches. The first sub-branch contain four bacterial isolates among which the isolates (Pv 7 burn) and (Pv 16 urine) reveled similarity (45%) and the isolates (Pv 11 vagina) and (Pv 1 wound) show similarity (53%). On the other hand, the second sub- branch contained four isolates (Pv 6 burn, Pv17 urine, Pv 18 urine, and Pv 4 wound) among which the isolates (Pv17 urine) and (Pv18 urine) with similarity (49%). However, the second sub-cluster contained two isolates (Pv 14 urin) and (Pv 5 burn) with similarity (10%). The similarity between deferent isolates like vagina and stool, urine and wound because nosocomial infection that taken from hospital patient .
By using RAPD primers, the isolate 11 showed the high similarity with isolate 1 (53%), followed by isolates 7 and 16 with similarity (45%), However, isolate 9 showed the lowest

similarity (8%). Moreover, the other isolated revealed intermediate percentages of similarity in between the lowest and highest percentage. It was found that most of isolates revealed low percentages of similarity and this assured the differences in the genetic backgrounds among 18 *P. vulgaris* clinical isolates under the present study.
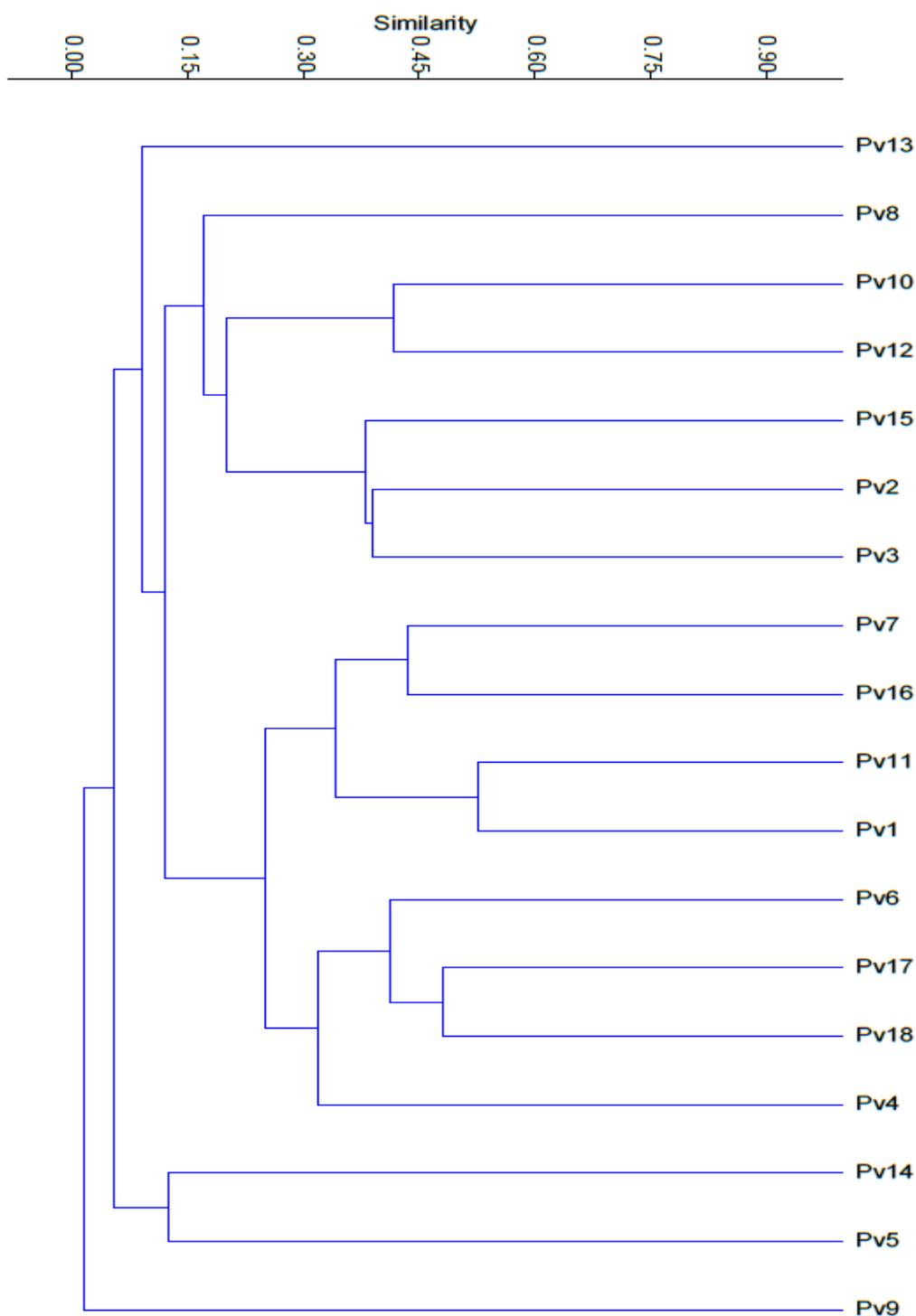

Figure 11: Dendrogram represented the genetic relationships among 18 *P. vulgaris* isolates using past3 cluster analysis from ten RAPD primer.

In this study, ten primers of RAPD-PCR revealed polymorphisms among *P. vulgaris* collected from different medical samples, either in the variable genetic similarities of each isolate with the others or in the occurrence of amplified fragments. The low and narrow variations because of the structure of the 10-mer primers RAPD and the genomic structure of the *P. vulgaris* species. The variation of genetic similarities values of the 18 isolates with other obviously showed the divergent genetic backgrounds of these isolates with their DNA polymorphisms patterns. Results of the present work revealed that the 18 isolates were different genetically. Additionally, primers (*OPA11)* and (*OPA-13*) exhibited high polymorphisms. [24] were displayed polymorphisms amongst 29 different isolates of *P. mirablis* from Brazil generating 86 bands, 51 of which were polymorphic. It was suggested that the polymorphisms between isolates due to that bio-typing often lacks power of discrimination because of variations and random mutations in gene expression that can modify biologic properties of bacteria.

The current study on RAPD analysis revealed that, there were some bands which are common in all the samples and some were not evident. In the present study, we have found that the genetic diversity in *P. vulgaris* depend on occurrence of mutants and source of isolation. DNA bands patterns to have a practical meaning in the areas of population biology, epidemiology and medicine, specific DNA bands should be allied to mutation, virulence genes and host origins.

However, the data in the present study confirm the wide genotypic diversity of *P. vulgaris* from various clinical samples. It is to note that, there was no correlation between the observed strain variability and the sample from which the isolates originated.

Although, the isolates located in the same cluster are isolated from different source but the results of RAPD give one important evidence on the closed relative of these isolates.

So, the present study reveals that, *P. vulgaris* is not specific for infections. This clearly shows that, one can't use the same drug for a particular infection caused by *P. vulgaris* because of their variation in DNA polymorphism. Therefore further study on antibiotic sensitivity and sequence analysis would help to devise and prescribe a better drug for the future.

The application of PCR based RAPD primers has been played a major tool in finding out the relationship between the various species of microorganisms [28]. The profile of amplification products depends on the combination between template and primer and is reproducible for any given combination.

It has been observed that bacterial isolates of interest have not only the same identity, but also high genetic similarities, even when isolated from different source

Effect of N-acetyl-cysteine on biofilm formation
Bacteria that are fixed inside a biofilm show strong tolerance to antibiotics and resistance to the immune system have a relative to planktonic bacteria [29]. The ability to form biofilm promotes the development and chronicity of infections. In order to study attachment and colonization of bacteria, a various of methods, direct and indirect have been developed, the micro-titer plate assay (biofilm assay) is the most used techniques for formation of biofilm. The results for production biofilm of *P. vulgaris* show that different potential capacity to form biofilm, P. vulgaris was found biofilm about 8(44.44%) are strong former and the 2(11.11%) are mild former and 8(44.44%) in weak former of biofilm formation, as showed in Table (3).

Table 3: biofilm formation by *P. vulgaris*

Bacterial isolates (no.)	Biofilm strong	moderate	weak	% of biofilm formation
18	8	2	8	55.55%

The result of this study was correlated with result obtained by [30] were found that *P. vulgaris* was able to produce biofilm at 42.59%. also this study correlated with other similar study done by [31] who reported 43% of isolates as non/weak biofilm producers and 57% as biofilm producers. According to the data obtained in this study, the presence of strong or moderate biofilm will confer bacteria to adhere strongly to the site of infection. However, weak positive may express the bacteria may be under stress condition or the growth is weak that made the biofilm weak or cannot be produced.

On the other hand, the effect of NAC on biofilm formation was studied; it was found that, the production biofilm of *P. vulgaris* show different potential capacity to form biofilm after added N-acetyl-cysteine (10%), the result of this study demonstrated effecting, reduce the viability of biofilm formation. The biofilm formation was reduced as moderate to (5.55%), weak (94.44%) and no strong. the result was demonstrated that the biofilm production altered from (55.55%) to (5.55%) as showed in table(4).The result of biofilm formation in present study indicate a reduced production of biofilm in *P. vulgaris* when addition or treated with NAC.

Table 4: Effect of NAC on biofilm formation

Bacterial isolates (no.)	Biofilm strong	moderate	Weak	% of biofilm formation
18	0	1	17	5.55%

N-acetyl-cysteine is generally non-toxic and can be tolerated at high doses, with a high favourable risk-to-benefit ratio with a reduced rate of adverse events [32]. Several studies have been shown that N-acetyl-cysteine reduces extracellular polysaccharide matrix production and inhibits adhesion of bacteria, decreases formation of biofilm, disrupt mature biofilm and the cell viability of a variety of Gram+ve and Gram-ve bacteria [33][34].

The activity of N-acetyl-cysteine in promoting dispersal of preformed biofilms could be related either to a direct effect of NAC in affecting biofilm matrix architecture or to perturbation of microbial physiology [35][36]. Also, N-acetyl-cysteine plays a role in biofilm destruction due to its

mucolytic properties [37][35] by cleaving di-sulphide bonds which crosslink glycoproteins. The inhibition biofilm ability of *P. vulgaris* will ease the treatment of bacteria and decrease its virulence

Effect of NAC and green tea on urease activity
It has been observed that increase NAC concentration result in decreased urease expression as shown in fig (12). So this results is identical to result obtained by [38] have demonstrate that NAC exhibits the effect against *Proteus* inhibiting the activity of urease.
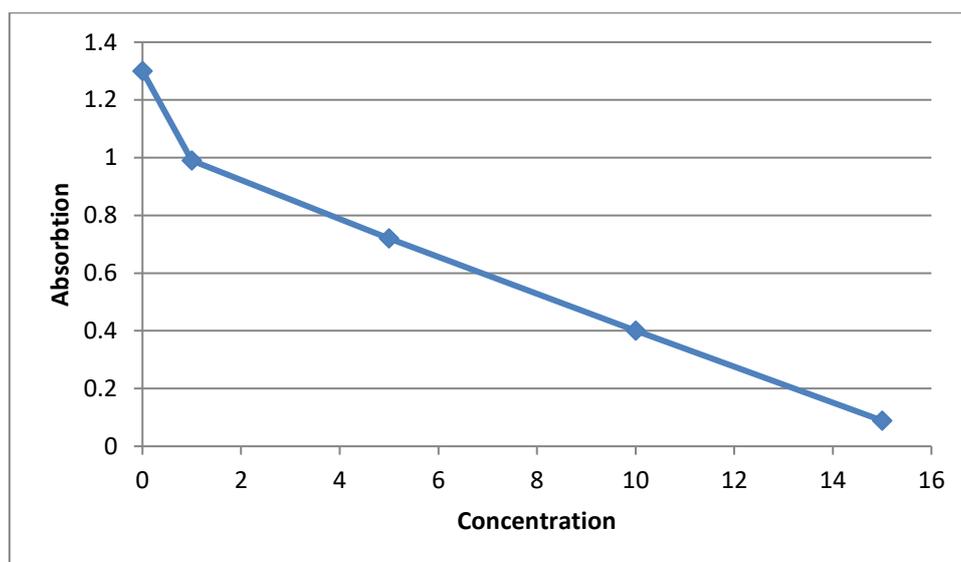

Figure 12: Effect of NAC on urease

On the other hand, the results were demonstrate that increase green tae concentration lead to inhibits the activity of urease-bacterially produced enzyme as shown in fig(13). The results shown NAC is more effective on urease than green tea.
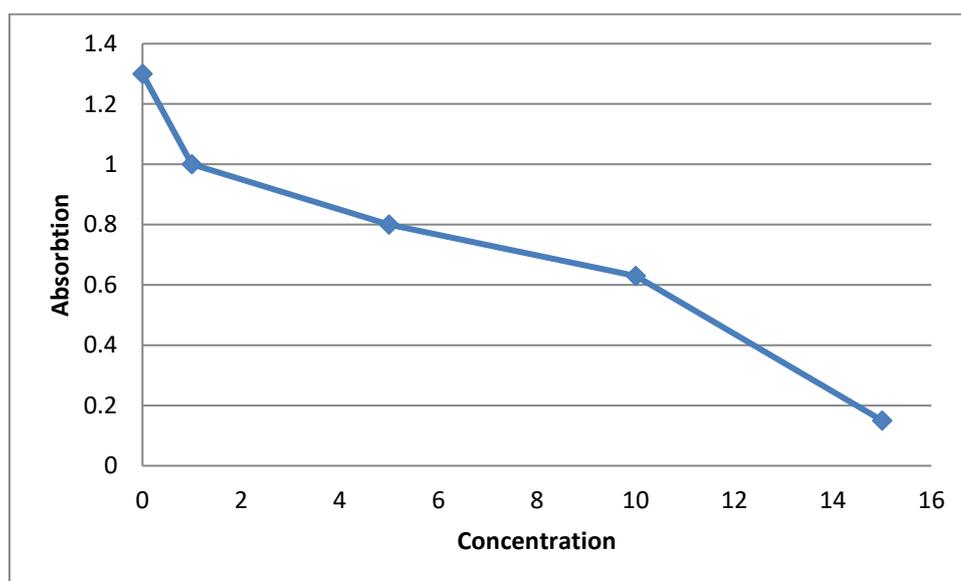

Figure 13: Effect of green tea on urease

Specific compounds that inhibition of can give a precious addition for the treatment of bacterial infectious diseases

due to urease-producing bacteria. Although several inhibitors of the urease enzyme is safe, more effective and it

considered important to regulate infections result from bacteria produce urease [39].

The N-acetyl-cysteine had the ability to inhibit production of urease. Based on the previous result, it was found the urease was inhibited by thiol compound present in N-acetyl-cysteine [13][40]. The present study was done by using N-acetyl-cysteine due to the presence of thiol compound in N-acetyl-cysteine which may effect on the activity of urease.

On the other hand, an aqueous extract of green tea leaves provides a good source of active beneficial materials which are advantageous to human [41]. Green tea is non toxic, safe and having no side effects after use. [42]

Urease activity from *Proteus vulgaris* was inhibited by increasing concentration of green tea. These results were suggested that inhibitors used in inhibition of urease may be beneficial for the treatment of bacterial infectious diseases.

CONCLUSION

Genotype of *P. vulgaris* by using RABD-PCR technique from different sample shown *P. vulgaris* strain are not specific for infection. NAC has the ability for the inhibition of biofilm formation by *P. vulgaris*. On the other hand, NAC and green tea showed varying inhibitor activity against urease enzyme

CONFLICT OF INTEREST

None

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