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Detection the Strains of Carbapenem Resistant Pseudomonas aeruginosa Isolated from Burn Infection and the Variety of Protein Forming bla_{NDM-1} Alleles

Conflict of interest: nothing to declare.
Authors' contribution: Sarah Khudhur – conceptualization, data curation, investigation, methodology, project administration, software, validation, writing – original draft and writing – review & editing; Munaff Abd Al-Abbas – conceptualization, data curation, methodology, supervision, validation, visualization, writing – original draft and writing – review & editing; Yahya Abbas – conceptualization, data curation, methodology, supervision, validation, visualization, writing – original draft and writing – review & editing.

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Abstract

Introduction. Random amplified polymorphic DNA (RAPD) is a technique used in the laboratory to detect polymorphism and slight genetic differences between closely related species and construct the genetic dendrogram among variety species to represent the relationships between the strains in the same species.
Purpose. Detect the Carbapenem resistant Pseudomonas aeruginosa strains isolated from infected burn and to determine the variety of protein forming bla_{NDM-1} alleles.
Materials and methods. Identical, closely related and unrelated strains were identified. The RAPD bands of each strain were quantified in base pairs (bp) according to the ladder's bands, the RAPD pattern data for all strains were processed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm developed. The sequence of nucleotides was converted to an amino acid sequence by translate the nucleotide sequence into protein sequence.
Results. Twenty-eight P. aeruginosa isolates that have identical patterns of antibiotic resistance were divided into 9 groups based on their resistance genes. Group A showed strain 26 and 28 were identicals, group B showed strain 7, 8 and 12 identicals, group C showed the strains 51, 37, 43 and 53 not related and group D showed the strains 5 and 19 considered not related and the other groups (E, F, G, H and I) each has a noval result. Carbapenem bla_{NDM-1} allele in 19 – P. aeruginosa and 23 – P. azotoformans have mutation alter two amino acids leading to change the protein structure, in the same vein, allele in 23 – P. azotoformans but with one amino acid changing.
Conclusions. The distribution of the same strains of P. aeruginosa among individuals indicating the ability of this strain for transmission and Some mutations were active because it changing the amino acid following the protein structure resulting a difference in antibiotic susceptibility.
Keywords: carbapenem, RAPD, protein, P. aeruginosa, infectious disease

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Выявление штаммов Pseudomonas aeruginosa, устойчивых к карбапенемам, выделенных из очагов ожоговой инфекции, и разнообразия белков, образующих аллели bla_{NDM-1}

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Резюме

Введение. Метод случайной амплификации полиморфной ДНК (RAPD) используется в лабораторных условиях для выявления полиморфизма и незначительных генетических различий между близкородственными видами и построения генетической дендрограммы среди различных видов, отражающей родственные связи между штаммами одного вида.
Цель. Выявить штаммы Pseudomonas aeruginosa, устойчивые к карбапенемам, выделенных из инфицированных ожогов, и определить разнообразие белок-образующих аллелей bla_{NDM-1}.
Материалы и методы. Были идентифицированы идентичные, близкородственные и неродственные штаммы. RAPD-полосы каждого штамма количественно определялись в парах оснований в соответствии с полосами на шкале. Данные о паттернах RAPD для всех штаммов обрабатывались с использованием разработанного алгоритма невзвешенного парного группового анализа со средним арифметическим (UPGMA). Последовательность нуклеотидов была преобразована в аминокислотную последовательность путем трансляции нуклеотидной последовательности в белковую.
Результаты. Двадцать восемь изолятов P. aeruginosa с идентичными профилями антибиотикорезистентности были разделены на 9 групп в зависимости от их генов резистентности. В группе A штаммы 26 и 28 были идентичны, в группе B – штаммы 7, 8 и 12, в группе C штаммы 51, 37, 43 и 53 не были родственными, в группе D штаммы 5 и 19 считались неродственными, а в остальных группах (E, F, G, H и I) результаты были новыми. Аллель карбапенема bla_{NDM-1} у 19 – P. aeruginosa и 23 – P. azotoformans имеет мутацию, изменяющую две аминокислоты, что приводит к изменению структуры белка. Аналогично, аллель у 23 – P. azotoformans, но с изменением одной аминокислоты.

Выводы. Распространение одних и тех же штаммов P. aeruginosa среди особей указывает на способность этого штамма к передаче, а некоторые мутации были активными, поскольку они изменяли аминокислоту в структуре белка, что приводило к разнице в восприимчивости к антибиотикам.
Ключевые слова: карбапенем, RAPD, белок, P. aeruginosa, инфекционное заболевание

■ INTRODUCTION

Random amplified polymorphic DNA (RAPD) is performed by using short primer of 10 bases, containing 30–70% of GC content with low annealing temperature, the short primer can pair to the template in a multi-locus with a different size of band and numbers measuring by electrophoresis [1]. RAPD is fast, cheaper and can perform to detect the strains of variety bacterial species without sequence of nucleotides [2]. The detection of the strains level of bacteria is important to determination the source of the bacterial infection, furthermore, to detect the suitable drug for treatment [3].

Carbapenems are beta-lactam antibiotics containing a beta-lactam ring and a five-membered ring, which is unsaturated and contains a carbon atom instead of a sulfur atom, thus differing from the penicillin ring [4, 5]. This unique molecular structure has remarkable stability towards most beta-lactamases including extended-spectrum beta-lactamases [6]. Substitutions of a few amino acids in a protein sequence resulting from genetic mutations alter the enzyme's structure, greatly broadening the spectrum of antibiotics susceptible to hydrolysis. Rapid mutations are possible, and in certain instances, microorganisms have developed antibiotic resistance while undergoing treatment [7]. In general, the position of a single nucleotide alteration predominantly dictates the occurrence of a mutation, a heterozygous mutation arises when a modification in the base sequence modifies a codon, substituting one amino acid in the protein with another, the severity of the heterozygous mutation is contingent upon the location and characteristics of the substituted amino acid. Mutations in amino acids that are conserved among proteins in phylogenetically related species typically impact the active site or the tertiary structure of the protein [8, 9].

■ MATERIALS AND METHODS

Sample collection

Out of 57 swabs obtained from patients with burn infections from the previous study (under publishing / Khudhur et al., 2025), 28 P. aeruginosa were identified by 16S rDNA sequencing for testing RAPD.

Random amplified polymorphic DNA (RAPD)

The RAPD primer sequence was 5-ACGGGAGCAA-3 and 5-GGCTGCAATG-3 Nielsen et al. [10]. The total volume of 25 µl comprised 12 µl of Go Taq Green master mix (Promega, USA), 7 µl of Nuclease-Free water (Bioneer, Korea), 4 µl of DNA template, and 2 µl of primer. The thermocycler (Bioneer, Korea) conditions for amplification included an initial denaturation at 95 °C for 5 minutes, followed by 45 cycles of denaturation at 94 °C for 1 minute, annealing at 37 °C for 1 minute, and extension at 72 °C for 2 minutes, concluding with a final extension at 72 °C for 5 minutes. A 100 bp DNA ladder (Promega, USA) was

utilized alongside 1.5% agarose powder, 100 ml of TBE buffer, and 0.5 µg/ml of ethidium bromide. Agarose gel electrophoresis was carried out to find the identical P. aeruginosa strain bands under a UV transilluminator (Wisd, Korea). All isolates' RAPD band distances were determined using Microsoft Word based on the ladder's bands and the results were imported into the "Unweighted pair group method with Arithmetic mean" (UPGMA) programmed to display as a dendrogram [11].

Data analysis

Identical, closely related and unrelated strains were identified. The RAPD bands of each strain were quantified in base pairs (bp) according to the ladder's bands as per Olorunfemi et al. [12], the RAPD pattern data for all strains were processed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm developed and refined by Garcia-Vallve and Puigbo [11]. Within, RAPD patterns of individual strains were compared based on the index of similarity between samples Chansiripornchai et al. [13], developing a mathematical model by computing a similarity matrix converting similarity coefficients into a distance matrix (with a Distance Matrix value of "0.000" signifying identical strains) and performing clustering to generate a dendrogram from a collection of variables in order to investigate genetic variation, particularly in challenging or closely related RAPD patterns. This sort of computational analysis facilitates direct comparisons without the necessity of counting bands, which is particularly crucial following resolution loss due to manuscript duplication through photocopying [14].

Drawing 3D shape of carbapenem protein

1. The sequence of nucleotides was converted to an amino acid sequence by translate the nucleotide sequence into protein sequence (http://reverse-complement.com/translate-protein/ROOT).
2. The correct sequence of amino acids was determined for each carbapenem gene with comparison to the Escherichia coli Ec7 strain from GenBank.
3. The Phyre 2 V.2.0 program and Swiss-Model program were used to draw the 3D shape of proteins and determine the different amino acids (https://swissmodel.expasy.org/interactive).

Statistical analysis

One-way ANOVA were performed to evaluate the differences among the studied tests using SPSS version 17.0, $P \leq 0.05$ were considered as statistically significant [15].

■ RESULT

Bio typing P. aeruginosa by antibiotic resistant genes

Twenty eight P. aeruginosa isolates that have identical patterns to carbapenems antibiotic resistance from the previous study were divided into 9 groups based on their resistance genes: group A of 9 isolates having 3 identical genes (bla_{OXA-48}, bla_{NDM-1} and OmpA), group B of 8 isolates having 2 genes (bla_{OXA-48} and bla_{NDM-1}), group C of 4 isolates contain 2 genes (bla_{OXA-48} and OmpA), group D of 2 isolates contain 4 genes (bla_{OXA-48}, bla_{NDM-1}, bla_{VIM} and OmpA) and the other groups each has a noval result (Table 1).

Table 1
Biotyping P. aeruginosa according to the antibiotic resistant genes

Antibiotic resistant P. aeruginosa, n=28		bla_{OXA-48}	bla_{NDM-1}	bla_{VIM}	bla_{KPC}	bla_{IMP}	OmpA	Groups
1	20	+	+	–	–	–	+	A
2	21	+	+	–	–	–	+	
3	25	+	+	–	–	–	+	
4	26	+	+	–	–	–	+	
5	28	+	+	–	–	–	+	
6	29	+	+	–	–	–	+	
7	30	+	+	–	–	–	+	
8	36	+	+	–	–	–	+	
9	39	+	+	–	–	–	+	
10	2	+	+	–	–	–	–	B
11	7	+	+	–	–	–	–	
12	8	+	+	–	–	–	–	
13	12	+	+	–	–	–	–	
14	24	+	+	–	–	–	–	
15	27	+	+	–	–	–	–	
16	31	+	+	–	–	–	–	
17	42	+	+	–	–	–	–	
18	51	+	–	–	–	–	+	C
19	37	+	–	–	–	–	+	
20	43	+	–	–	–	–	+	
21	53	+	–	–	–	–	+	
22	5	+	+	+	–	–	+	D
23	19	+	+	+	–	–	+	
24	4	–	+	+	–	–	+	E
25	14	–	+	–	–	–	+	F
26	50	–	–	–	–	–	+	G
27	52	+	–	–	–	–	–	H
28	56	–	+	–	–	–	–	I

P. aeruginosa strains

Each above of identical group result (A, B, C and D) was tested separately for RAPD. Group A showed strain No. 26 identical to strain No. 28 (Fig. 1, 2).

Group B showed strain No. 7 identical to clinical strains No. 8 and 12 (Fig. 3. 4).

Group C showed the strains No. 51, 37, 43 and 53 were consider not related (Fig. 5, 6).

Group D showed the strains No. 5 and 19 considered not related (Fig. 7, 8).

Protein structure of Carbapenem (bla_{NDM-1}) allele

The previous study of sequence and analysis of bla_{NDM-1} gene for the three species of Pseudomonas including 19 – P. aeruginosa, 23 – P. azotoformans and Acinetobacter baumannii showed three different alleles distributed in these genera according to point mutations (under publishing / Khudhur et al., 2025). Following, changing the amino acids (Fig. 9). The first allele is represented by isolate No. 19 – P. aeruginosa that differed from isolate No. 16 – Acinetobacter baumannii and P. azotoformans (identical to the type

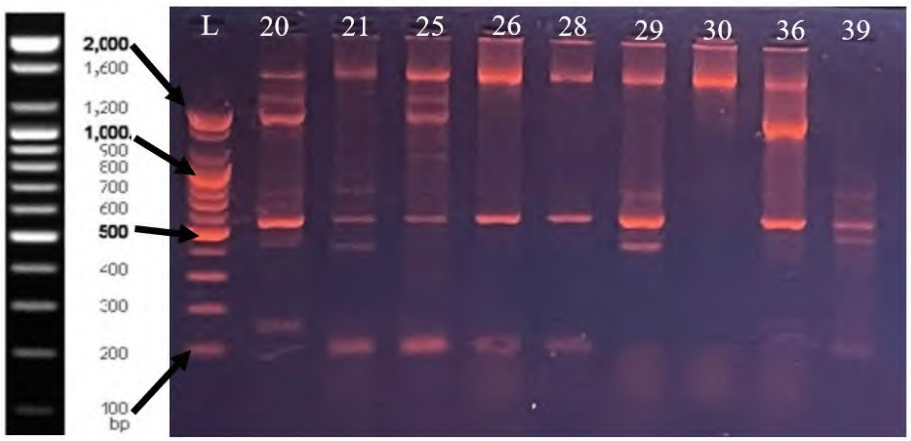

Fig. 1. Agarose gel electrophoresis (1.5%) showed RAPD patterns of P. aeruginosa. Lane L: 25/100 bp Mixed DNA ladder. Lines 20, 21, 25, 26, 28, 29, 30, 36 and 39: bacterial isolates. Strains No. 26 and 28 were identical

strain Escherichia coli Ec7) by one amino acid (Valine instead of Lysine respectively). The protein shape or structure was changed (Fig. 10). The second one is represented by isolate No. 19 – P. aeruginosa that differed from isolate No. 16 and 23 (identical to Escherichia coli Ec7) by one amino acid (Methionine instead of Lysine respectively). The protein shape or structure was changed (Fig. 8). Finally, is represented by isolate No. 23 – P. azotoformans that differed from isolate No. 16 and 19 (identical to Escherichia coli Ec7) by one amino acid (serine instead of arginine respectively). The protein shape or structure was changed (Fig. 12).

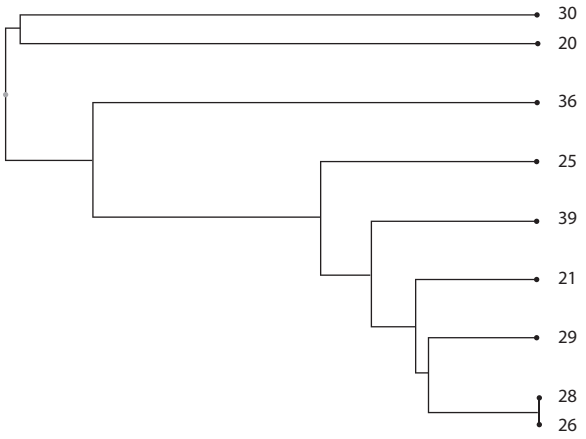

Fig. 2. Dendogram of P. aeruginosa strains (No. 20, 21, 25, 26, 28, 29, 30, 36 and 39) performed by variables related to RAPD bands using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. Strains No. 26 identical to No. 28

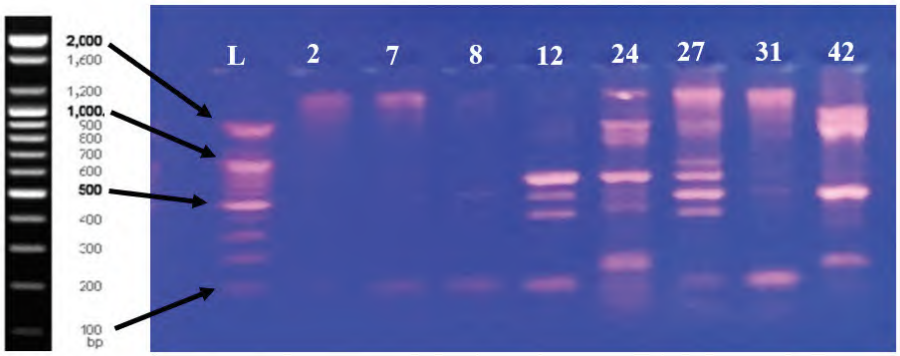

Fig. 3. Agarose gel electrophoresis (1.5%) showed RAPD patterns of P. aeruginosa. Lane L: 25/100 bp Mixed DNA ladder. Lines 2, 7, 8, 12, 24, 27, 31 and 42: bacterial isolates. Strains No. 7, 8 and 12 were identical

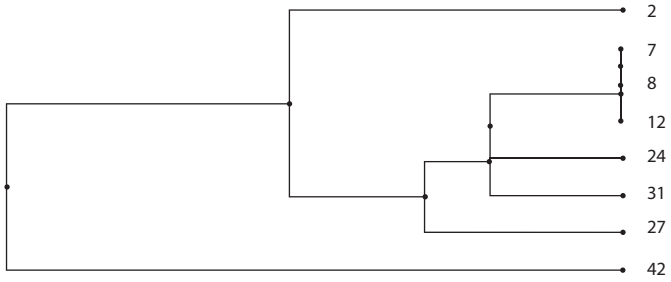

Fig. 4. Dendogram of P. aeruginosa strains (No. 2, 7, 8, 12, 24, 27, 31 and 42) performed by variables related to RAPD bands using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. Strains No. 7, 8 and 12 were identical

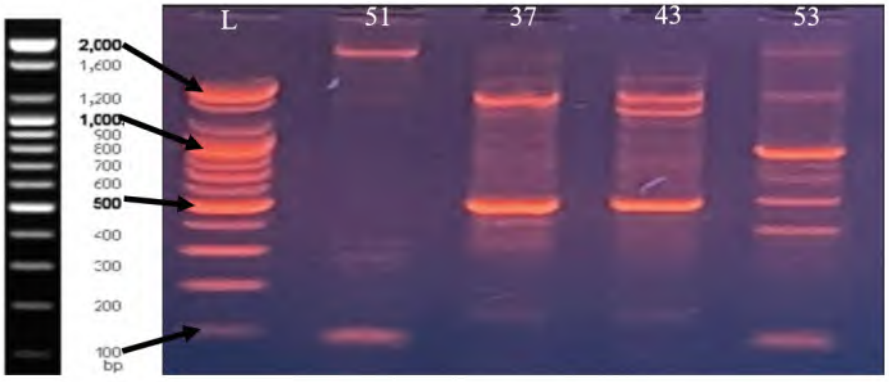

Fig. 5. Agarose gel electrophoresis (1.5%) showed RAPD patterns of P. aeruginosa. Lane L: 25/100 bp Mixed DNA ladder, lines: 51, 37, 43 and 53 bacterial isolates (no identical strains)

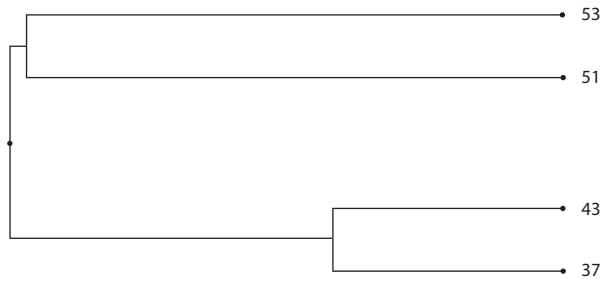

Fig. 6. Dendogram of P. aeruginosa strains (37, 43, 51 and 53) performed by variables related to RAPD band using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm (no identical strains)

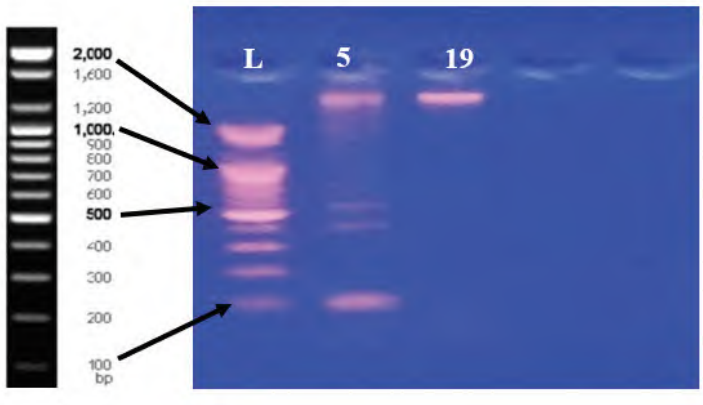

Fig. 7. Agarose gel electrophoresis (1.5%) showed RAPD patterns of P. aeruginosa. Lane L: 25/100 bp Mixed DNA ladder, lines: bacterial isolates No. 5 and 19 (no identical strains)

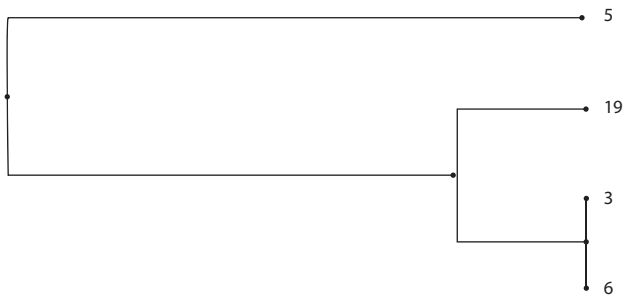

Fig. 8. Dendogram of P. aeruginosa strains (5 and 19) performed by variables related to RAPD band using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm (no identical strains)

Note: * 3 and 6 are out groups (control).

Fig. 9. The amino acid sequence of 3 NDM-1 alleles including isolate No. 19 – P. aeruginosa (Valine and Methionine), No. 23 – P. azotoformans (serine), and No. 16 (without amino acid changing) with their type strain Escherichia coli Ec7

■ DISCUSSION

Rapid molecular approaches such as RAPD-PCR have exhibited significant specificity and sensitivity in characterizing bacterial isolates, hence facilitating epidemiological investigations on the spread of P. aeruginosa isolates within a certain environment Mahdi et al. [16]. The 28 P. aeruginosa isolates were underused for screening toward the 6 antibiotics genes. Group A showed 2 strains (26 and 28) from two patients have identical strains of P. aeruginosa strain, group B showed 3 strains (7, 8 and 12) from 3 patients were identical, these samples can be identical because they belong to the same ancestor and they were taken from the same source from which the sample was isolated thus could will be transmitted from one patient to another. Group D have two identical strains (3 and 6). However, the presence of identical strains indicates that the entire strain has been transmitted between people, not just the gene, which indicates the risk of the infection spreading more widely. Moreover, the ability of a strain to transport among different patients could be responsible of many pathogenic gene transes missed by Horizontal gene transfer [17]. While, group C which have 4 different strains they were all non-identical even they isolated from the same source, making these strains very dangerous because resistance genes move quickly between different strains. The allele in 19 – P. aeruginosa have two amino acids altered were substituted from Valine instead of Lysine and Methionine instead of Lysine leading to the change in the protein structure.

On the other hand, one amino acid was substituted from Serine instead of Arginine in 23 – P. azotoformans leading to the change in the protein structure. Despite the fact that there is a mutation in the allele of 16 – A. baumannii, the amino acid does not change, and as a result, the protein does not alter. The important explanation of this mutations is some gene or point mutation doesn't change the amino acid calling silent mutation

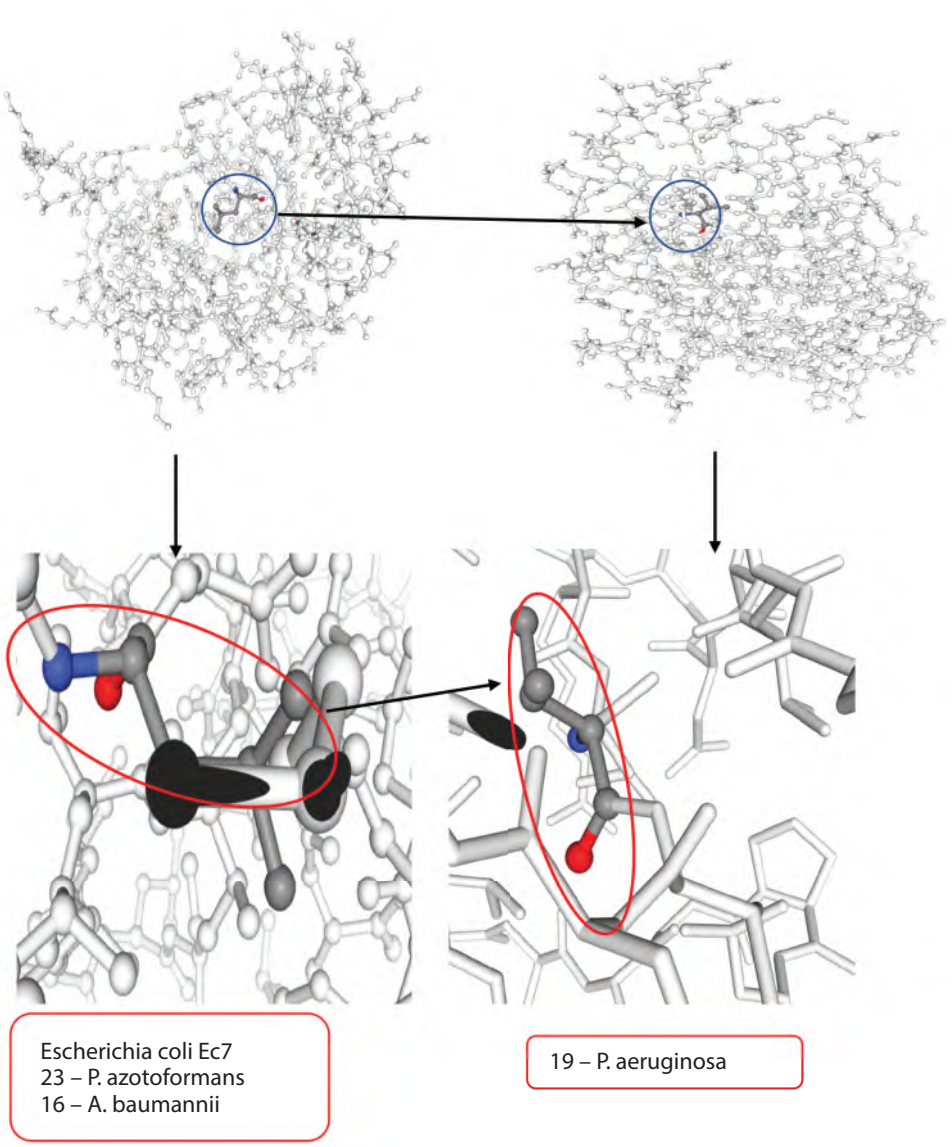

Fig. 10. Three-dimensional shapes of bla$_{NDM-1}$ alleles by Swiss-Model program NDM-1 protein of Escherichia coli Ec7, 23 – P. azotoformans and 16 – A. baumannii isolate comparing with NDM-1 protein of No. 19 – P. aeruginosa mutated by valine instated of lysine was changed in the shape

as recorded by study of Zankari et al. [18]. But the dangerous mutation when it has the ability to change the amino acid to another producing a different protein, then a different action. However, mutations that impact fewer essential portions of the protein typically have consequences that are not very large, and the activity may still be significant.

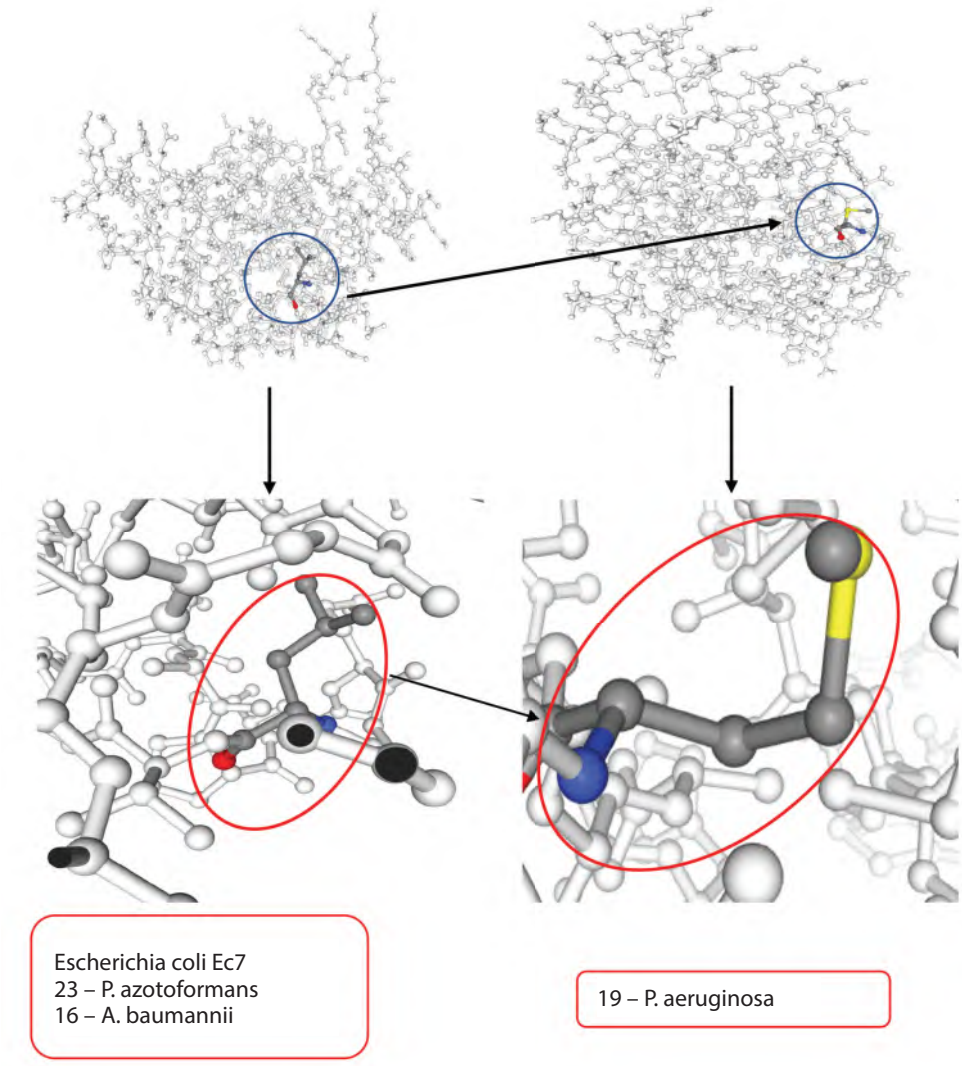

Fig. 11. Three-dimensional shapes of bla$_{NDM-1}$ alleles by Swiss-Model program NDM-1 protein of Escherichia coli Ec7, 23 – P. azotoformans and 16 – A. baumannii isolate comparing with NDM-1 protein of No. 19 – P. aeruginosa mutated by methionine instated of lysine was changed in the shape

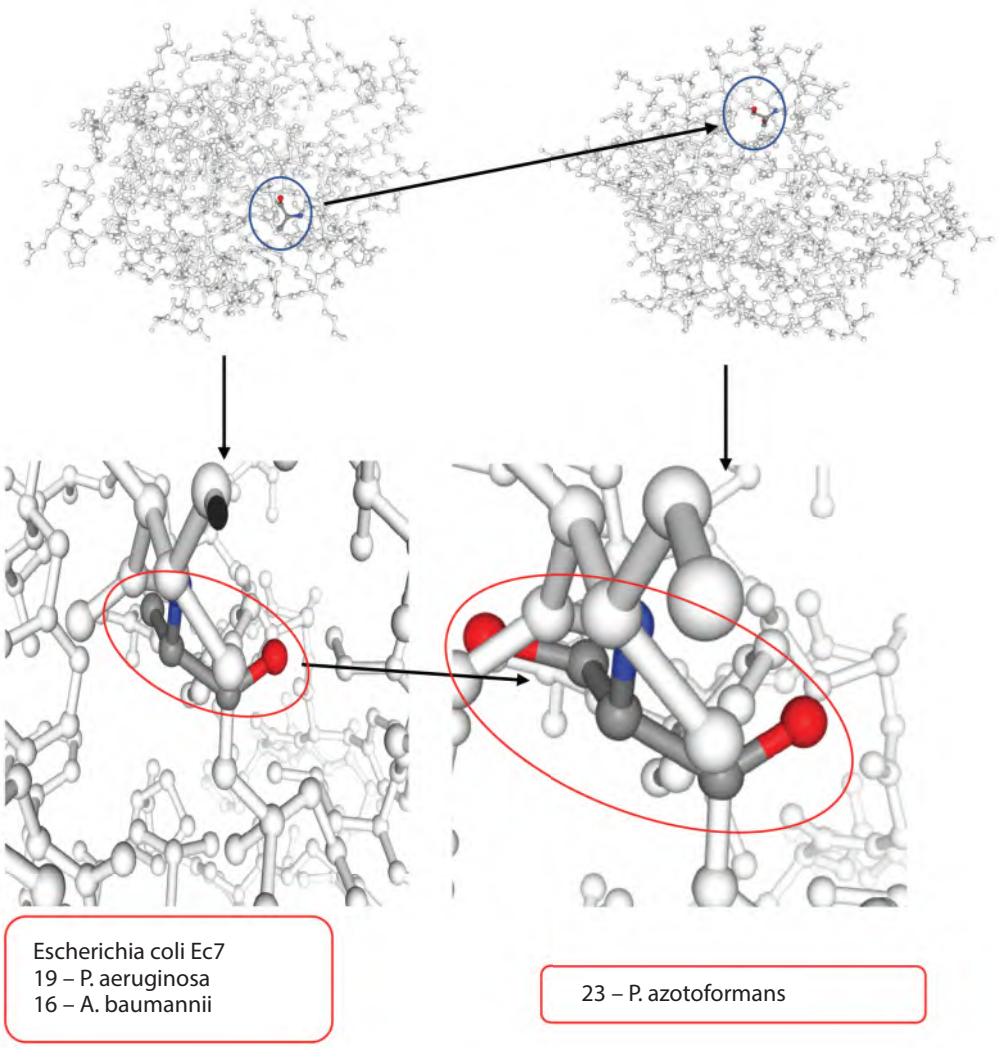

Fig. 12. Three-dimensional shapes of bla_{NDM-1} alleles by Swiss-Model program NDM-1 protein of Escherichia coli Ec7, 19 – P. aeruginosa and 16 – A. baumannii isolate, and NDM-1 protein of No. 23 – P. azotoformans mutated by serine instated of arginine was changed in the shape

■ CONCLUSIONS

The distribution of the same strains of P. aeruginosa among individuals indicating the ability of this strain for transmission and Some mutations were active because it changing the amino acid following the protein structure resulting a difference in antibiotic susceptibility.

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