

## Changes in Outer Membrane Proteins of Benzalkonium Chloride Adapted *Pseudomonas Aeruginosa* and Mutations in GyrA and ParC Genes

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### Abstract

**Background:** Benzalkonium Chloride (BC) is widely used in hospitals, industry and cosmetics. Adaptation of *Pseudomonas aeruginosa* to BC was increased. This adaptation may lead to the emergence of cross-resistance to other disinfectants and antibiotics. Little attention has been focused on the resistant mechanisms.

**Aim of Study:** Examination of mutations in the Quinolone-resistance-Determining Region (QRDR) of GyrA and ParC genes and alterations of Outer-Membrane Proteins (OMPs) in Benzalkonium Chloride (BC)-adapted *Pseudomonas aeruginosa* isolates.

**Material and Methods:** GyrA and ParC genes of the BC-adapted *P. aeruginosa* isolate and the wild-type strain ATCC 15442 were amplified by Polymerase-Chain-Reaction (PCR) and sequenced. OMPs of these isolates were also analyzed by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) in the presence and absence of permeabilizer disodium Ethylenediamine-Tetraacetate (EDTA) to investigate the variations.

**Results:** The results manifested a single mutation in both GyrA (Thr-83-Ile) and ParC (Ser-87-Leu). In SDS-PAGE, one band with molecular weight of 52.23kDa was detected in all samples. Seven bands with molecular weights of 71.44, 38.48, 36.01, 33.03, 26.17, 21.14 and 6.35kDa were stable in both ATCC 15442 and treated isolate. Also, seven bands with molecular weights of 120.83, 90.89, 79.72, 44.58, 28.91, 16.12 and 12.49kDa were newly induced in the treated isolate.

**Conclusions:** These results revealed that mutations in GyrA and ParC genes, and alteration of OMPs contributed to the adaptation of *P. aeruginosa* to BC, co-operating with efflux pump over-expression.

**Key Words:** *Benzalkonium chloride – Pseudomonas aeruginosa – GyrA, ParC – Mutations – Outer membrane proteins – SDS – PAGE.*

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### Introduction

**RECENTLY**, disinfectants were used indiscriminately for cleanliness purposes. This led to bacterial adaptation to those products such as Quaternary Ammonium Compounds (QACs) and resistance of *P. aeruginosa* isolates was frequently observed and adapted to QACs [1,2].

Benzalkonium Chloride (BC) is the most common QACs used. Their influence may not be limited to their areas of use, because it ultimately reaches the environment via wastewater and remains there for a long period, due to their poor biodegradability [3].

*Pseudomonas aeruginosa* is one of the most important nosocomial pathogens [4]. It causes several systemic infections, especially in immunosuppressed patients, and in patients with bed ulcers and severe burns [5-7]. *Pseudomonas aeruginosa* readily adapted to BC and consequently reducing their utility. The main mechanisms of resistance are mutations in the regulatory genes of the multidrug efflux pumps. Other mechanisms include mutations in GyrA and ParC genes that encode DNA Gyrase and topoisomerase IV respectively and the innate impermeability of the membrane [8,9].

High level of QACs exposure may induce a stress response leading to the expression of the SOS response, which could facilitate mutations in the Quinolone Resistance Determining Region (QRDR); the region where mutations arise in GyrA and ParC genes [3,10,11]. Mutations in QRDR, resulting in amino acid substitutions, alter the

target protein structure and leading to FQ/BC resistance [12,13].

Like FQ, BC target DNA GyrAse, and topoisomerase IV by inhibiting their control of supercoiling within the cell. Which results in impaired DNA replication (at lower concentrations) and cell death (at lethal concentrations) [14,15].

Bacterial adaptation due to the exposure to antimicrobial agents includes some alterations of the outer membrane proteins [16]. Outer Membrane Proteins (OMPs) are necessary to cell viability because they are in the interface between the intra and extracellular parts of the cell and perform essential functions in various cellular processes such as cell adhesion, metabolites and ion transport, and endocytosis host immune responses [17]. Thus, OMPs are very important for pharmacological action and represent potential targets for vaccine development [18,19].

### Material and Methods

This work was conducted at microbiology lab (B) in National Organization for Drug Control and Research along the period of 2015/2018.

#### *Bacterial isolates:*

*Pseudomonas aeruginosa* ATCC 15442 (purchased from Microbiologica USA) was used as a wild-type strain and isolate no. 87 (BC-adapted *P. aeruginosa* isolate) [20].

#### *PCR amplification and DNA sequencing of GyrA and ParC genes:*

The GyrA and ParC genes were PCR amplified with the pairs of primers as shown in (Table 1), according to Salma et al., [21], the PCR mixture, containing a 100ng of chromosomal DNA, 0.5 gM concentration of each primer, and Dream taq™ green PCR Master Mix (Thermo-Fisher Scientific, USA) was heated for 3 minutes at 95°C. Followed by 32 cycles of 30 seconds at 94°C; 30 seconds at 58°C (GyrA) and 55°C (ParC); and 60 seconds at 72°C, with final 10 minutes extension step at 72°C.

Table (1): Primers used in GyrA and ParC genes detection.

| Gene | Product size | Sequence                      |
|------|--------------|-------------------------------|
| GyrA | 287          | Forward : GTGTGCTTTATGCCATGAG |
|      |              | Reverse : GGTTTCCTTTTCCAGGTC  |
| ParC | 267          | Forward : CATCGTCTACGCCATGAG  |
|      |              | Reverse : AGCAGCACCTCGGAATAG  |

The PCR products were purified with the DNA purifying kit (Thermo Fischer, USA) according to manufacturer's instructions. The sample was proc-

essed with DNA sequencing kit (Thermo Fischer, USA) and sequenced by an automatic DNA sequencer (Applied Biosystem- serial no. G: 43A: 39T: 30C: 41) in order to analyze mutation in genes. Sequencing was carried out in Biotechnology lab, VACSERA, Dokki, Egypt. The DNA sequences were aligned and homology searches were performed using BLAST program ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)) and the translation to amino acids was performed using ExpASY translate <https://web.expasy.org/translate/>

#### *Phenotypic detection of outer membrane permeability:*

Assessment of BC-adapted isolate for outer membrane permeability was performed by determination of the Minimum Inhibitory Concentration (MIC) value for Benzalkonium Chloride (BC) in the presence and absence of Disodium ethylenediaminetetraacetate (EDTA).

EDTA was used as a permeabilizer which chelates divalent cations that stabilize molecular interactions in the outer membrane causing disruption of Outer Membrane Proteins (OMPs). To avoid the effect of EDTA on bacterial growth, it was used at a concentration of  $1/4$  MIC and less. A four-fold reduction in the BC MIC or more in the presence of EDTA indicates outer membrane reduced permeability activity [22].

As described previously the MICs of EDTA carried out by using microtiter method [23] and the MIC of BC-EDTA were determined by agar dilution method according to CLSI (M100-S25) guidelines [24]. To evaluate the effect of the combinations, the Fractional Inhibitory Concentration (FIC) index was calculated [25].

#### *Outer membrane proteins analysis:*

##### *Extraction of outer membrane proteins:*

Outer Membrane Proteins (OMPs) of both wild-type strain ATCC 15442, treated isolate (isolate no. 87 treated with sub-MIC of EDTA + BC) and untreated isolate (isolate no. 87 without treatment) were extracted according to Yehia et al. [26].

Bacterial inoculum was incubated at 37°C for 48 hours in 100mL of Tryptic Soya broth. The collected cells after centrifugation (6,000 Xg for 10 minutes at 4°C Sigma laboratory centrifuge 3k30, UK) were suspended in 3mL of HEPES (10mM, pH 7.4), and disrupted by sonication (FALC Italy UTA-60, 45 seconds at 50% output). Cell debris was discarded by centrifugation at

6,000Xg for 10 minutes at 4°C. Then the supernatant was added to 0.75mL of 2% N-lauroylsarcosine and kept at room temperature for 10 minutes. The detergent- solubilized OMPs were recovered by centrifugation at 100,000Xg for 1 hour (SorvallTM MTX 150 Micro-Ultracentrifuge, Thermo Fisher Scientific, 34,500rpm). Proteins pellet was resuspended in 3mL of 10mM HEPES (pH 7.4), incubated with 1 volume of sarkosyl for 20 minutes at room temperature, and recovered by ultracentrifugation as explained before. Finally, the pellet was resuspended in 1mL of 10mM HEPES and stored at -20°C.

*Protein content determination:*

The protein content of OMPs was determined as g/dL using colorimetric method (Biuret reagent-Spectrum Kit).

*Determination of molecular weight of OMPs bands:*

The molecular weight of OMPs bands was determined by using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Biorad) according to Laemmli [27]. The OMPs were solubilized in 0.05M Tris-HCl buffer (2.5% SDS, 5% 2-mercaptoethanol, 25% glycerol, and 0.003% bromophenol blue) and incubated at 100°C for 7 minutes before inoculation to the stacking gel (4%) using three concentration of separating gel (8, 10 and 12.5%). The gel was stained by Coomassie blue staining and the observed protein bands were analyzed by Gel Documentation Software (GelDocu Advanced Ver. 3.0) for the determination of their molecular weight.

**Results**

*Detection and sequencing of GyrA and ParC genes by PCR technique:*

The Quinolone Resistant Determining Region (QRDR) of GyrA (287-bp) and ParC (267-bp) was amplified for the wild-type strain ATCC 15442 and the BC-adapted isolate no. 87 using agarose gel electrophoresis to detect these genes Fig. (1) and then were sequenced for the gene mutation detection.

Figs. (2,4) showed the nucleotides sequence alignment of GyrA and ParC genes of wild-type strain ATCC 15442 and isolate no. 87. While Figs. (3,5) showed the amino acids alignment of GyrA and ParC genes of wild-type strain ATCC 15442 and isolate no. 87.

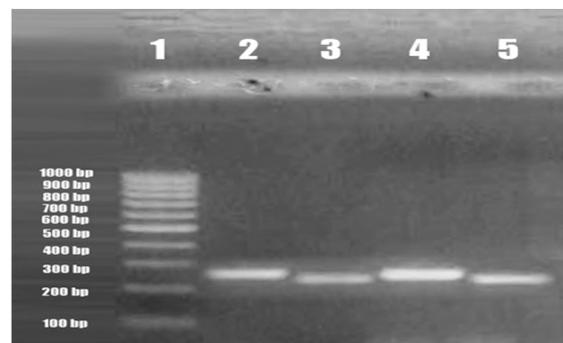


Fig. (1): Detection of GyrA and ParC genes by agarose gel electrophoresis of PCR-amplified products of the isolates was shown. Lane 1, DNA ladder; lanes 2 & 4 represent the amplified GyrA products for wild-type strain and the isolate no. 87 respectively and lanes 3 & 5 represent the amplified ParC products for wild-type strain and the isolate no. 87 respectively.

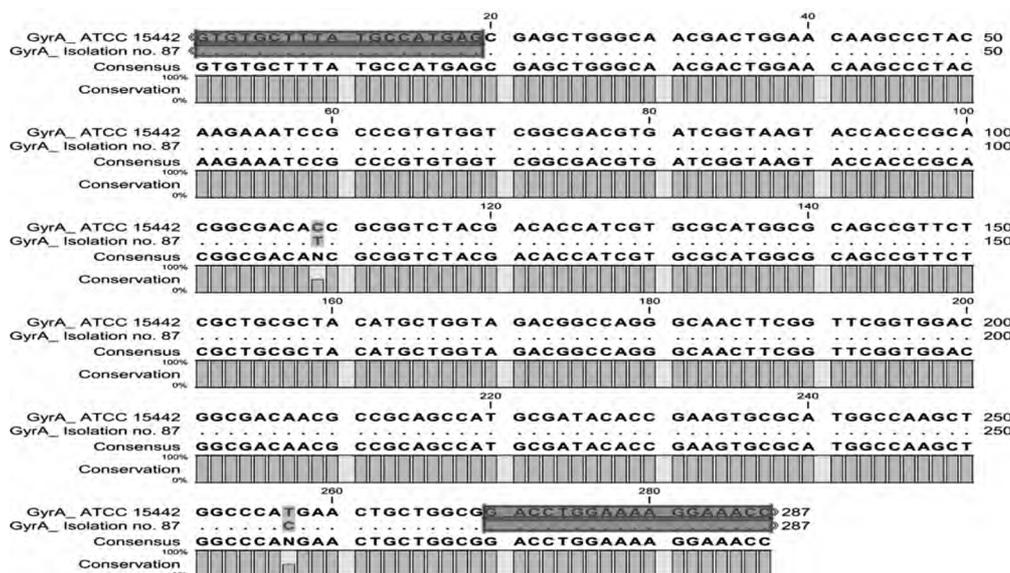


Fig. (2): Alignment of nucleotide sequences of the GyrA gene

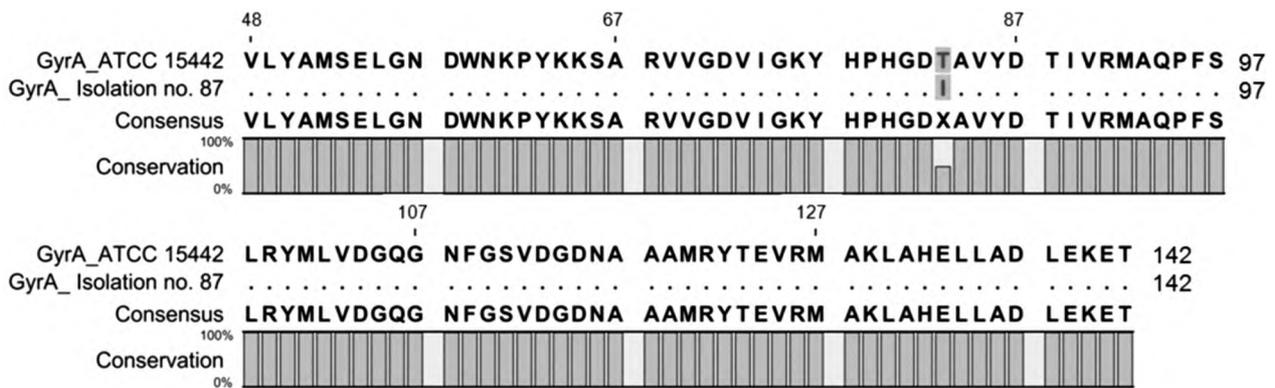


Fig. (3): Alignment of amino acid sequences of the GyrA gene.

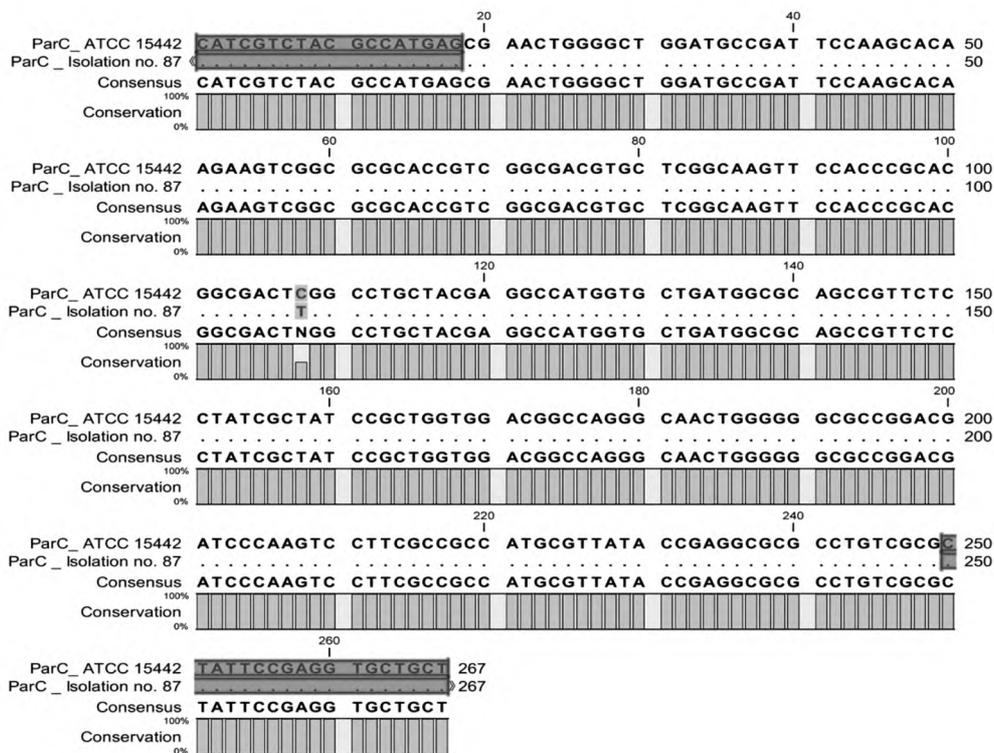


Fig. (4): Alignment of nucleotide sequences of the ParC gene.

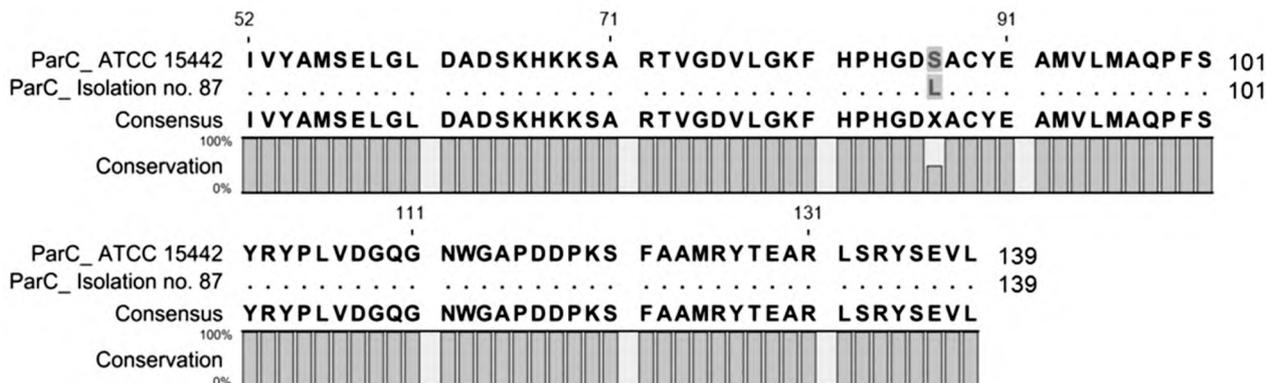


Fig. (5): Alignment of amino acid sequences of the ParC gene.

The obtained results in (Table 2), showed a single mutation in codon 83 of the GyrA gene and in codon 87 of the ParC gene, which substitutes the amino acid profile from Threonine to Isoleucine and Serine-to Leucine, respectively.

*Phenotypic detection of outer membrane permeability:*

Disodium Ethylenediaminetetraacetate (EDTA) was used for phenotypic detection of OMPs; generally, EDTA increased the effect of different antimicrobial agents as it reduced their MICs. The MIC of BC decreased in the presence of EDTA (512mg/L) from 2048 to 256mg/L.

Fractional inhibitory concentration index values in (Table 3), stated a synergistic antimicrobial activity of BC-EDTA (FIC <0.5) against the BC-adapted isolate (no. 87).

*Outer membrane proteins analysis:*

*Protein content determination:*

Results in (Table 4), illustrated the OMPs contents (g/dL) of the wild-type (ATCC 15442), isolate no. 87 with and without treatment. The results indicated that the OMPs contents were approximately constant in all samples.

*Determination of molecular weight of OMPs bands:*

The separation of OMPs on SDS-PAGE was done using three different concentrations of polyacrylamide gel (8, 10 & 12.5%). The gel was run three times for each concentration to confirm the results. Concentration 10% of polyacrylamide

gel gave the best separation of the OMPs for the samples.

Results in (Table 5) and Fig. (6), indicated the molecular weight, intensity and the amount (%) of outer membrane proteins extracted from ATCC 15442, 87 (treated) and 87 (untreated) at conc. 10%, respectively using SDS-PAGE analysis.

Table (2): Type of point mutation in GyrA and ParC genes of P. aeruginosa isolate no. 87.

| Gene | Codon | Mutation     | Amino acid substitution |
|------|-------|--------------|-------------------------|
| GyrA | 83    | ACC ---- ATC | Threonin -- Isoleucine  |
| ParC | 87    | TCG ---- TTG | Serine -- Leucine       |

Table (3): Fractional inhibitory concentration index (FIC index) of benzalkonium chloride and EDTA.

| Identified resistant isolates | Type of biocide | MIC of biocide |                | FIC of each biocide | FIC index | Outcome |
|-------------------------------|-----------------|----------------|----------------|---------------------|-----------|---------|
|                               |                 | Alone          | In combination |                     |           |         |
| Isolate no. 87                | BC              | 2048           | 256            | 0.125               | 0.169     | Synergy |
|                               | EDTA            | 11630          | 512            | 0.044               |           |         |

BC : Benzalkonium Chloride.  
EDTA : Disodium Ethylenediaminetetraacetate

Table (4): Outer membrane content of the wild-type strain (ATCC 15442), untreated and treated isolate no. 87.

| Sample name   | OMP content (gm/dL) |
|---|---------------------|
| Wild-type strain ATCC 15442                             | 4.10                |
| Isolate no.87 (untreated)                               | 4.33                |
| Isolate no. 87 (treated with 256mg/l BC + 256mg/l EDTA) | 4.12                |

Table (5): Molecular weights, intensity and amount % of extracted outer membrane proteins of the tested P. aeruginosa ATCC15442, BC-adapted isolate no. 87 treated with sub-MICs of EDTA and BC and without treatment.

| Band | Marker  |           |      |      | ATCC 15442 |           |      |       | 87 (Treated) |           |      |       | 87 (Untreated) |           |      |      |   |   |   |   |
|------|---------|-----------|------|------|------------|-----------|------|-------|--------------|-----------|------|-------|----------------|-----------|------|------|---|---|---|---|
|      | *Mol Wt | Intensity | Area | %    | Mol Wt     | Intensity | Area | %     | Mol Wt       | Intensity | Area | %     | Mol Wt         | Intensity | Area | %    |   |   |   |   |
| 1    | 245     | 747384    | 2640 | 6.14 | -          | -         | -    | -     | -            | -         | -    | -     | -              | -         | -    | -    |   |   |   |   |
| 2    | 165     | 812592    | 2640 | 6.67 | -          | -         | -    | -     | -            | -         | -    | -     | -              | -         | -    | -    |   |   |   |   |
| 3    | 125     | 889944    | 2640 | 7.31 | -          | -         | -    | -     | 120.83       | 671760    | 2160 | 4.56  | -              | -         | -    | -    |   |   |   |   |
| 4    | 93      | 869880    | 2640 | 7.14 | -          | -         | -    | -     | 90.89        | 673704    | 2160 | 4.57  | -              | -         | -    | -    |   |   |   |   |
| 5    | 72      | 1042008   | 2640 | 8.56 | 72.95      | 751192    | 2480 | 9.07  | 79.72        | 683208    | 2160 | 4.64  | -              | -         | -    | -    |   |   |   |   |
|      |         |           |      |      |            |           |      |       | 71.44        | 695736    | 2160 | 4.72  | -              | -         | -    | -    |   |   |   |   |
| 6    | 57      | 986568    | 2640 | 8.1  | 52.23      | 2315328   | 6944 | 27.97 | 52.23        | 1403568   | 4536 | 9.53  | 52.23          | 1237768   | 4216 | 100  |   |   |   |   |
|      |         |           |      |      |            |           |      |       | 44.58        | 1721736   | 5184 | 11.7  | -              | -         | -    | -    |   |   |   |   |
| 7    | 42      | 934560    | 2640 | 7.67 | 39.11      | 753672    | 2480 | 9.1   | 38.48        | 718200    | 2160 | 4.87  | -              | -         | -    | -    |   |   |   |   |
|      |         |           |      |      |            |           |      |       | 36.32        | 678032    | 2232 | 8.19  | 36.01          | 744120    | 2160 | 5.05 | - | - | - | - |
|      |         |           |      |      |            |           |      |       | 32.44        | 1525696   | 4960 | 18.43 | 33.3           | 1875960   | 5400 | 12.7 | - | - | - | - |
| 8    | 31      | 986832    | 2640 | 8.1  | 26.92      | 755160    | 2480 | 9.12  | 28.91        | 732456    | 2160 | 4.97  | -              | -         | -    | -    |   |   |   |   |
|      |         |           |      |      |            |           |      |       | 26.17        | 1151712   | 3672 | 7.82  | -              | -         | -    | -    |   |   |   |   |
| 9    | 24      | 1040160   | 2640 | 8.54 | 20.56      | 744248    | 2480 | 8.99  | 24.14        | 902232    | 2808 | 6.12  | -              | -         | -    | -    |   |   |   |   |
| 10   | 18      | 1032240   | 2640 | 8.48 | -          | -         | -    | -     | 16.12        | 683856    | 2160 | 4.64  | -              | -         | -    | -    |   |   |   |   |
| 11   | 15      | 991320    | 2640 | 8.14 | -          | -         | -    | -     | 12.49        | 684288    | 2160 | 4.64  | -              | -         | -    | -    |   |   |   |   |
| 12   | 8       | 996600    | 2640 | 8.18 | 7.17       | 755160    | 2480 | 9.12  | 6.35         | 1390824   | 4536 | 9.44  | -              | -         | -    | -    |   |   |   |   |
| 13   | 3.5     | 84928     | 2640 | 6.9  | -          | -         | -    | -     | -            | -         | -    | -     | -              | -         | -    | -    |   |   |   |   |

\*Mol Wt: Molecular Weight.

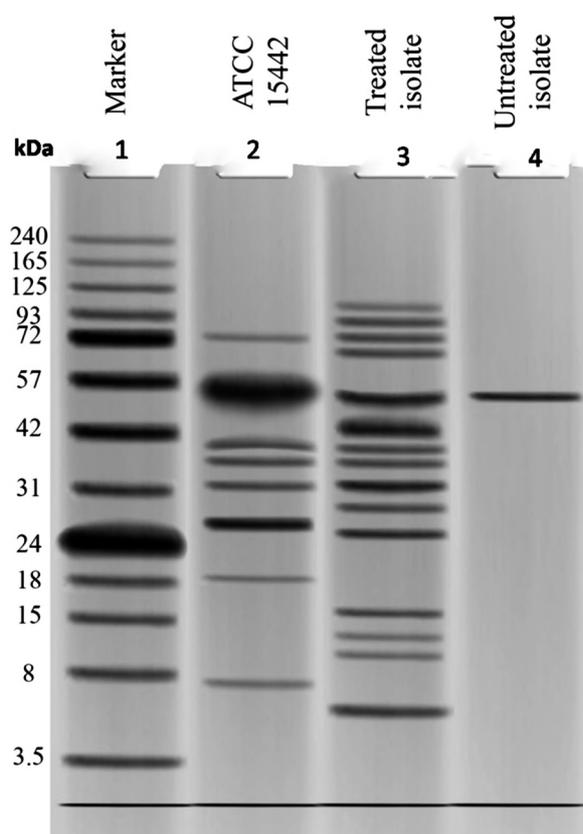


Fig. (6): Outer membrane proteins banding pattern of the wild-type (ATCC 15442), isolate no. 87 with and without treatment. Lane 1: Marker, lane 2: ATCC 15442, lane 3: Isolate 87 (treated), and lane 4: Isolate 87 (untreated) with 256mg BC/256mL EDTA.

Results showed one band with molecular weight of 52.23kDa was detected in both treated and untreated isolate, as well as in wild-type strain ATCC 15442. In addition, seven bands with molecular weights of 71.44, 38.48, 36.01, 33.03, 26.17, 21.14 and 6.35kDa were stable in both ATCC 15442 and treated isolate. Also, seven bands with molecular weights 120.83, 90.89, 79.72, 44.58, 28.91, 16.12 and 12.49kDa were detected in the treated isolate.

On the other hand, the results illustrated the intensity and total amount (%) of OMPs detected. It indicated that the amount of band 52.23 decreased in untreated isolate compared to ATCC strain 15442 and treated isolate. The down-regulation of the protein bands in untreated isolate may be due to the high adaptation to benzalkonium chloride.

Results of mutual bands illustrated that the intensity of most OMPs increased in treated isolate compared to wild-type strain ATCC 15442 except two bands with molecular weight of 71.44 and 38.48kDa.

## Discussion

The results of mutations detected in GyrA and ParC genes are consistent with previous reports on clinical isolates of *P. aeruginosa* adapted to BC [28-31].

This was also consistent with the results of other studies as Gorgani et al., [32] who revealed that mutations related to antibiotic resistance were detected in codons 83 and 87 of GyrA, also Salma et al., [21] reported that among 38 isolates, 11 were susceptible, 22 were resistant, and 5 were intermediate to ciprofloxacin. They found that 19 (50%) of these strains had a mutation in the GyrA gene (Thr 83 Ile), the ciprofloxacin-sensitive strains had no mutations in the sequence area examined. Also they found that 81.8% of the isolates that were resistant to ciprofloxacin had a mutation in the GyrA gene. So Thr-83 → Ile was shown to be the chief mechanism of fluoroquinolones resistance. This observation confirmed that the DNA gyrase was the primary target for fluoroquinolone resistance in the clinical isolates of *P. aeruginosa* [30].

Tohidpour et al., [28] reported that the substitution of Threonine (polar) to Isoleucine (non-polar) increase FQ/BC-adaptation due to its influence in the GyrAse-FQ/BC interaction by the loss of necessary enzyme-BC contacts.

In a previous study, isolate no. 87 has a mutation in the MexR (regulatory gene MexAB-OprM efflux system) in the codon 126 (substituting amino acid Valine to Glutamic acid) [20]. It was supposed that mutation in mexR gene may not reduce the susceptibility of the bacteria alone but when it co-occurs with a mutation on the GyrA gene [8,29].

The 52.23 protein band was previously reported as oprM protein which is responsible for the efflux pump activity [33,34]. In addition, seven bands with molecular weights of 71.44, 38.48, 36.01, 33.03, 26.17, 21.14 and 6.35kDa were similar to those published earlier as opr C, E, F, R, G, H and I, respectively [19,35-37].

OprC and OprE are anaerobic-inducible porins, although it is conceivable that low levels of these small-channel porins may be present in wild-type cells [33,38-40]. OprF is a major outer membrane protein in *P. aeruginosa*, which have a nonspecific porin function and binds to the underlying peptidoglycan [39,41-43]. Clinical isolates of *P. aeruginosa* that are multiply antibiotic resistant and deficient in the major outer membrane protein OprF have been obtained [19,33,39,44,45]. OprR is an outer membrane associated protein with molecular mass 26kDa. The actual function of OprR is not yet clear

[1]. OprG was first investigated by Yates et al., [46]. Various groups suggested a link between the presence of OprG and resistance of *P. aeruginosa* to antibiotics because increased resistance to norfloxacin, kanamycin, and/or tetracycline was associated with the disappearance or downregulation of OprG [19,36,47,48]. While OprH is a gated porin for divalent cations [33,49]. Lipoproteins, like OprI has been shown to play a role in many fundamental cellular processes and in the pathogenesis of *Pseudomonas aeruginosa* strains, where OprI was down-regulated in adapted *P. aeruginosa* for BC [50-52]. As an integral OM component, OprI plays a role in cell shape and membrane fluidity maintenance [53]. According to Linares et al., [54], the exposure of *P. aeruginosa* to tobramycin and ciprofloxacin also promoted the down-regulation of the OprI gene.

Also, seven bands with molecular weights 120.83, 90.89, 79.72, 44.58, 28.91, 16.12 and 12.49kDa were detected in the treated isolate. The induction of protein with high molecular weight could be attributed to iron-limited conditions (ED-TA is a chelating agent) [22,26,55].

#### Conclusion:

Finally, the continuous increase in the prevalence of BC-adapted isolates could be attributed to the widespread use and misuse of these products. This resistance is mainly due to over-expression of efflux pump activity, the presence of mutations in the quinolone targets (DNA gyrase and topoisomerase IV) and alteration of OMPs.

This study complements the identification of adaptation mechanisms of *P. aeruginosa* to BC and revealed that mutations in the GyrA and ParC genes and alternation in OMPs co-operated with efflux pump over-expression in the adaptation of *P. aeruginosa* to BC, which also confirm the presence of cross-resistance between BC and FQ genetically.

The present results also confirm previous findings regarding to obtain a combination of Benzalkonium Chloride plus efflux pump inhibitor or outer membrane permeabilizer which can be used pharmaceutically to restore disinfectant activity and minimize further developments of resistant strains.

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## التغيرات في بروتينات الغشاء الخارجى والطفرات فى جينات ParC و GyrA لميكروب سودوموناس اريجونوزا المقاوم لكلوريد بنزالكونيوم

يستخدم كلوريد البنزالكونيوم على نطاق واسع فى المستشفيات، الصناعة ومستحضرات التجميل. زادت نسبة تكييف ميكروب سودوموناس اريجونوزا لكلوريد البنزالكونيوم. قد يؤدي هذا التكيف إلى ظهور مقاومة للمطهرات الأخرى والمضادات الحيوية، وقد ركز القليل من الإهتمام على آليات المقاومة. تم الكشف عن الطفرات فى منطقة مقاومة الكينولون (QRDR) للجينات ParC و GyrA عن طريق إستخدام ال PCR والتتابع التسلسلى للأحماض الأمينية لهذه الجينات للعزلة المقاومة لكلوريد البنزالكونيوم (٨٧) وعزله ال ATCC 15442. أيضا تم الكشف عن التغيرات البروتينية للغشاء الخارجى للعزلة ٨٧ وال ATCC 15442 بإستخدام ال SDS-PAGE فى وجود وغياب مادة ال EDTA التى تزيد من نفاذية الغشاء الخارجى. أظهرت النتائج طفرة واحدة فى كل من GyrA (Thr-83-Ile) و ParC (Ser-87-Leu). فى SDS-PAGE، تم الكشف عن بروتين واحد بوزن جزيئى ٥٢.٢٣ كيلو دالتون فى جميع العينات. وجد سبع بروتينات بأوزان جزيئية من ٣٦.٠١ و ٣٨.٤٨ و ٧١.٤٤ و ٢٦.١٧ و ٢١.١٤ و ٦.٢٥ كيلو دالتون مستقرة فى كل من ATCC 15442 والعزل المعالجة بال EDTA والغير معالجة. أيضا، تم تحفيز ٧ بروتينات بالأوزان الجزيئية ١٢٠.٨٣، ٩٠.٨٩، ٧٩.٧٢، ٤٤.٥٨، ٢٨.٩١، ١٦.١٢ و ١٢.٤٩ كيلو دالتون فى العزلة المعالجة. فى الختام، قد ساهمت الطفرات فى جينات ParC و GyrA، والتغير ببروتينات الغشاء الخارجى فى تكييف السودوموناس اريجونوزا لكلوريد البنزالكونيوم، بالتعاون مع الإفراط فى ضخ مضخات الطرد.