

## *Pseudomonas* Species Isolated from Camels: Phenotypic, Genotypic and Antimicrobial Profile

ADEL M. ABDELRAHMAN<sup>1</sup>, SAHAR R. MOHAMED<sup>1</sup>, SOLIMAN M. SOLIMAN<sup>2</sup>, SHERIF MAROUF<sup>3\*</sup>

<sup>1</sup>Bacteriology department, Animal Health Research Institute, Dokki, Giza, 12618, Egypt; <sup>2</sup>Department of Medicine and Infectious Disease, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt; <sup>3</sup>Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.

**Abstract** | One of the important livestock economies are camels which adapt with adverse environmental conditions and provide milk, meat, wool, hides, and skin. Gram-negative *Pseudomonas aeruginosa* is harbor multidrug antimicrobial resistance of camel has serious consequences for human health, so, this study aimed to characterized of *P. aeruginosa* especially extended spectrum  $\beta$ -lactamases (ESBL) producing one; phenotypic and genotypic. The isolates of *P. aeruginosa* were confirmed biochemically by a Vitek 2 compact system (bioMérieux). Suspected *P. aeruginosa* colonies carry ESBL were 56.6% by the double disc synergy test (DDST). Detection of virulence genes using *pslA*, *toxA* and *exoU* genes revealed that 29.4%, 23.5% and 17.6% respectively. Molecular detection of ESBLs encoding genes in *P. aeruginosa* recorded that *bla*TEM genes *bla*SHV and *bla*CTXM genes were detected in percentages of 64.7%, 47.0 % and 29.4%, respectively. Finally, ESBL *P. aeruginosa* showing multidrug antimicrobial resistance that detected by *mexR* gene.

**Keywords** | Camels, *P. aeruginosa*, ESBL, Antibiotic resistance, Virulence genes

**Received** | August 19, 2021; **Accepted** | October 05, 2021; **Published** | January 04, 2022

**\*Correspondence** | Sherif Abd Elmonam Marouf, Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt; **Email:** dr.sherif.marouf@gmail.com

**Citation** | Abdelrahman AM, Mohamed SR, Soliman SM, Marouf S (2022). *Pseudomonas* species isolated from camels: Phenotypic, genotypic and antimicrobial profile. Adv. Anim. Vet. Sci. 10(2): 219-225.

**DOI** | <http://dx.doi.org/10.17582/journal.aavs/2022/10.2.219.225>

**ISSN (Online)** | 2307-8316

## INTRODUCTION

In Middle East region, camels are important in the livestock economy by naturally resistant to adverse environmental conditions and most of the diseases commonly affecting livestock (Ismail et al., 2014). camels are the main source of meat and milk in many regions of the world, mainly in Africa and Asia, playing a crucial role in their economy. Therefore, as they are important food sources in semi-arid and arid zones the picture of dromedaries transformed from “ship of the desert” to a “food security livestock” species and the camel industry is in transition from nomadism to intensive production. Although this trend recognizes the economic value of this livestock species as a food source, it could also make camels an increasingly important source for zoonotic disease transmission to humans, especially in resource poor communities with improper sanitation and

medical access (FAO, 2019).

*Pseudomonas aeruginosa* causes different diseases in both livestock and companion animals as endometritis, otitis, hemorrhagic pneumonia, mastitis and urinary tract infections (Salomonsen et al., 2013). Bacteria adapt and acquire resistance from misuse and overuse of antibiotics in treatment of human illness, animal husbandry and antibiotics residues in agriculture leaves (CheeSanford et al., 2009). The increasing resistance of potentially pathogenic bacteria to multiple conventional antibiotics is an urgent problem in global public health (Strauß et al., 2015). The multiple-drug-resistant (MDR) *Pseudomonas* can be transmitted from different sources to humans and also to the environment through horizontal gene, the emergence and occurrence of MDR *P. aeruginosa* strains are growing in the world, leading to limited therapeutic

options (Breidenstein et al., 2011). Transmission of ESBL-producing gram-negative bacteria between food-producing animals and humans via direct contact or meat is supposed (Smet et al., 2010).

As few knowledges is available about *P. aeruginosa* in camel, this study aimed to investigate the ESBL producing *P. aeruginosa* from apparent healthy and diseased camels especially as this microorganism has the ability of producing multidrug resistant enzymes that could be easily disseminated in the community between livestock.

## MATERIALS AND METHODS

### SAMPLES

Two hundred and fifty nasal swabs collected from apparent healthy (150) and diseased camels (100) at different Cairo and Giza farms and abattoirs, then sent to the laboratory on the ice box for bacterial examination.

### CULTIVATION AND ISOLATION OF *P. AERUGINOSA*

Following culture of samples on cetrimide agar, the plates were incubated aerobically at 37°C for 24 hours. The Suspected colonies were picked up for morphological and biochemical identification (Quinn et al., 2004) as traditional method of identification and reinvestigated biochemically by Vitek2 compact system according to the manufacture structure (Biome'rieux, 2006; Sahar et al., 2014).

### DIFFERENTIATION BETWEEN ESBL AND NON ESBL BY DOUBLE DISK SYNERGY TEST METHOD (DDST)

*P. aeruginosa* isolates were phenotypically identified as ESBL by double disk synergy test as described by (Jarlier et al., 1988) method. A Mueller–Hinton agar was inoculated with standardized inoculum (corresponding to 0.5 McFarland tube) using a sterile cotton swab, then an amoxicillin clavulanic acid (AMC 30 µg) disk placed in the center of the plate 15 mm away from ceftriaxone (CRO 30 µg), ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg) and aztreonam (ATM 30 µg). The plate was incubated at 37 °C overnight. So, enhancement of the zone of inhibition of any one of the four drug disks toward amoxicillin–clavulanic acid suggested identification of extended-spectrum beta-lactamases (ESBL). *P. aeruginosa* ATCC 27853 was used as a control strain for a positive ESBL.

### ANTIMICROBIAL RESISTANCE TEST

Antimicrobial resistance test was conducted on ESBL producing *P. aeruginosa* strains using the Kirby-Bauer disk diffusion method (Bauer et al., 1966) by using Mueller–Hinton agar plates the antimicrobial susceptibility are measured according to the standard procedures of Clinical and Laboratory Standards Institute guidelines CLSI (2020). the antimicrobial susceptibility of *P. aeruginosa*

isolates was tested against different antimicrobial drugs of different classes: β-lactamas e.g Penicillin G (P 10µg), aztreonam (AT 30 µg), 3<sup>rd</sup> generation cephalosporin e.g cefotaxime (CTX30 µg), 4<sup>th</sup> generation cefepime (FEP 30 µg), Carbapenems e.g., imipenem (IPM 10 µg), meropenem (MEM 1 µg), Aminoglycosides e.g., gentamicin (GEN 10 µg), Quinolones e.g., ofloxacin (OFX 5 µg), Macrolides e.g., erythromycin (E15µg) and Sulfonamides e.g sulphamethoxazole/ trimethoprim (SXT 25 µg). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality controls.

### MOLECULAR IDENTIFICATION OF *P. AERUGINOSA*, VIRULENCE GENES, ESBLs ENCODING GENES AND MULTIDRUG RESISTANCE GENE BY POLYMERASE CHAIN REACTION (PCR)

DNA from samples was extracted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH). Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Primers used were supplied from Metabion (Germany) to detect *P. aeruginosa* 16S rDNA, ESBLs encoding genes (*bla*TEM, *bla*SHV and *bla*CTXM), virulence genes (*tox*A, *exo*U, and *psl*A) and multidrug resistance gene (*mex*R). Target genes, oligonucleotide primer sequences and the expected product size in different PCR assays are listed in Table 1. Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Appllichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A generuler 100 bp ladder (Fermentas, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

## RESULTS AND DISCUSSION

### PHENOTYPIC CHARACTERIZATION OF *PSEUDOMONAS* spp.

Thirty isolates from nasal swab samples of 250 camels with the percentage (12%) as shown in Table 2 were produced characteristic bright green color growth features

of *Pseudomonas* species on the cetrimide agar medium. All isolates were reinvestigated biochemically by GN card of Vitek 2 system (bioMérieux) and all isolate were confirmed as *Pseudomonas aeruginosa* as automated biochemical tests for *P. aeruginosa*.

### PHENOTYPIC DETECTION OF ESBL BY DOUBLE DISK SYNERGY TEST METHOD (DDST)

Phenotypic detection of ESBL by DDST revealed that 17 *P. aeruginosa* were ESBL producing *P. aeruginosa* isolates. So, a total percentage of ESBL was detected in a percentage

of 56.6% (17/30) from nasal swab isolates.

### ANTIMICROBIAL SUSCEPTIBILITY TESTING

The results of the antimicrobial susceptibility testing for the 17 ESBL *P. aeruginosa* shows a high-level resistance (100%) to 3<sup>rd</sup> generation cefotaxime, 4<sup>th</sup> generation cefepime, followed by carbapenem: Meropenem and imipenem (88.2%) and (82.3%) and penicillin (82.3%), gentamicin (76.4%), aztreonam (70.5%), erythromycin (29.5%), sulphamethoxazole/trimethoprim (29.5%), and highly sensitive for ofloxacin (100% sensitive) as shown in Table 3.

**Table 1:** Primer names, target genes, oligonucleotide primer sequences and the expected product size used in different PCR assay.

| Gene                          | Primer sequence 5'-3'                                                        | Amplified product (bp) | Reference                        |
|-------------------------------|------------------------------------------------------------------------------|------------------------|----------------------------------|
| <i>P. aeruginosa</i> 16S rDNA | GGGGGATCTTCGGACCTCA<br>TCCTTAGAGTGCCACCCG                                    | 956                    | Spilker <i>et al.</i> , 2004     |
| <i>exoU</i>                   | CCGTTGTGGTGCCGTTGAAG<br>CCAGATGTTACCGACTCGC                                  | 134                    | Winstanley <i>et al.</i> , 2005  |
| <i>pslA</i>                   | TCCCTACCTCAGCAGCAAGC<br>TGTTGTAGCCGTAGCGTTTCTG                               | 656                    | Ghadaksaz <i>et al.</i> , 2015   |
| <i>toxA</i>                   | TGTTGTAGCCGTAGCGTTTCTG<br>CGCTGGCCCATTCGCTCCAGCGCT                           | 396                    | Matar <i>et al.</i> , 2002       |
| <i>bla</i> TEM                | ATCAGCAATAAACCCAGC<br>CCCCGAAGAACGTTTTC                                      | 516                    | Colom <i>et al.</i> , 2003       |
| <i>bla</i> SHV                | AGGATTGACTGCCTTTTTC<br>ATTTGCTGATTTCGCTCG                                    | 392                    |                                  |
| <i>bla</i> CTX-M              | ATG TGC AGY ACC AGT AAR GTK ATG GC<br>TGG GTR AAR TAR GTS ACC AGA AYC AGC GG | 593                    | Archambault <i>et al.</i> , 2006 |
| <i>mexR</i>                   | GCGCCATGGCCCATATTCAG<br>GGCATTCGCCAGTAAGCGG                                  | 637                    | Sánchez <i>et al.</i> , 2002     |

**Table 2:** No. and % of *P. aeruginosa* isolated form camels' nasal swab.

| Samples                                                         | Number of samples | Number of positive isolates | Percentage |
|-----------------------------------------------------------------|-------------------|-----------------------------|------------|
| Nasal swab from apparent healthy camels                         | 150               | 8                           | 5.3%       |
| Nasal swab from diseased camels with respiratory manifestations | 100               | 22                          | 22.0%      |
| Total                                                           | 250               | 30                          | 12%        |

**Table 3:** Antibiotic resistance pattern of 17 ESBL *P. aeruginosa* isolates.

| Antibiotic class                         | Antibiotic                             | Sensitive |       | Resistance |       |
|------------------------------------------|----------------------------------------|-----------|-------|------------|-------|
|                                          |                                        | No.       | (%)   | No.        | %     |
| Quinolones                               | Ofloxacin (5 µg)                       | 17        | 100%  | 0          | 0%    |
| Aminoglycosides                          | Gentamicin (10 µg)                     | 4         | 23.5% | 13         | 76.4% |
| Sulfonamides                             | Sulphamethoxazole/trimethoprim (25 µg) | 12        | 70.5% | 5          | 29.5% |
| 4 <sup>th</sup> generation cephalosporin | Cefepime (30 µg)                       | 0         | 0%    | 17         | 100%  |
| 3 <sup>rd</sup> generation cephalosporin | Cefotaxime (30 µg)                     | 0         | 0%    | 17         | 100%  |
| β-lactamase                              | Penicillin G (10µg)                    | 3         | 17.6% | 14         | 82.3% |
|                                          | Aztreonam (30 µg)                      | 5         | 29.4% | 12         | 70.5% |
| Carbapenems                              | Imipenem (10 µg)                       | 3         | 17.6% | 14         | 82.4% |
|                                          | Meropenem (1.0 µg)                     | 2         | 11.7% | 15         | 88.2% |
| Macrolides                               | Erythromycin (15 µg)                   | 12        | 70.5% | 5          | 29.5% |

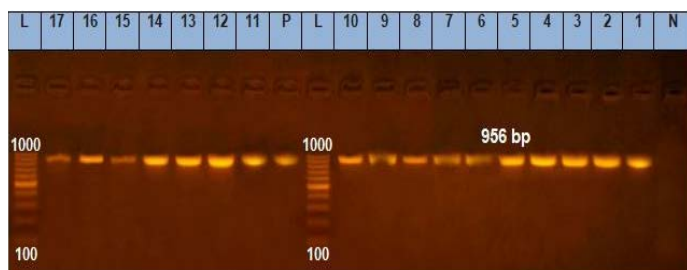


**Table 4:** Virulence genes, ESBL encoding genes, Multidrug resistance gene profile of *P. aeruginosa* isolates.

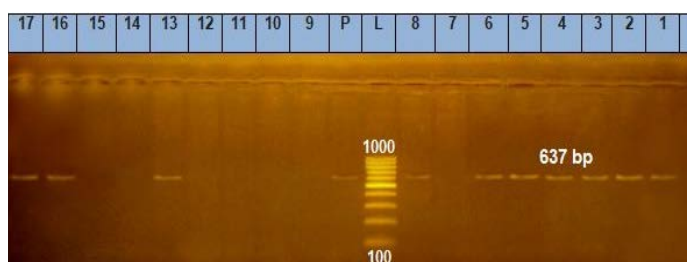
| Genes / isolate no.           | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | Total +ve | %     |
|-------------------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|-----------|-------|
| <i>P. aeruginosa</i> 16S rDNA | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | 17        | 100%  |
| <i>pslA</i>                   | - | + | + | - | - | - | - | - | - | -  | -  | -  | +  | -  | +  | -  | +  | 5/17      | 29.4% |
| <i>toxA</i>                   | - | - | + | - | - | - | - | - | - | +  | -  | -  | +  | -  | -  | +  | -  | 4/17      | 23.5% |
| <i>exoU</i>                   | - | - | + | - | - | - | + | + | - | -  | -  | -  | -  | -  | -  | -  | -  | 3/17      | 17.6% |
| <i>bla</i> TEM                | + | + | - | + | + | + | + | + | - | +  | -  | -  | +  | -  | -  | +  | +  | 11/17     | 64.7% |
| <i>bla</i> SHV                | + | - | + | + | - | - | - | + | - | -  | +  | -  | -  | -  | +  | +  | +  | 8/17      | 47.0% |
| <i>bla</i> CTX-M              | + | + | - | - | + | - | - | - | - | -  | -  | -  | -  | -  | +  | -  | +  | 5/17      | 29.4% |
| <i>mexR</i>                   | + | + | + | + | + | + | - | + | - | -  | -  | -  | +  | -  | -  | +  | +  | 10/17     | 58.8% |

## MOLECULAR DETECTION OF ESBL ENCODING GENES, VIRULENCE GENES, MULTIDRUG RESISTANCE GENE

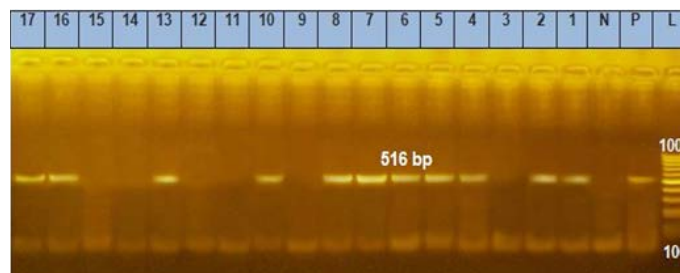
As shown in Table 4, all 17 ESBL (100%) isolates had confirmed to 16S rDNA gene (Figure 1). PCR screening of genes encoding ESBL revealed the amplification of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes in tested isolates as follow eleven out of 17 ESBL-positive isolates had *bla*<sub>TEM</sub> (64.7%), eight had *bla*<sub>SHV</sub> gene (47.0%) and five carried *bla*<sub>CTX-M</sub> gene (29.4%) as shown in (Figures 3, 4, 5, respectively). According to virulence genes profile of *toxA*, *exoU* and *pslA*, the results revealed that *pslA* gene presented in a percentage of (5/17) 29.4%, *toxA* found in an incidence of (4/17) 23.5% and *exoU* (3/17) 17.6% as shown in (Figures 6, 7, 8 respectively). Ten out of 17 *P. aeruginosa* harbored multidrug resistance gene *mexR* in percentage 58.8% as shown in Figure 2.



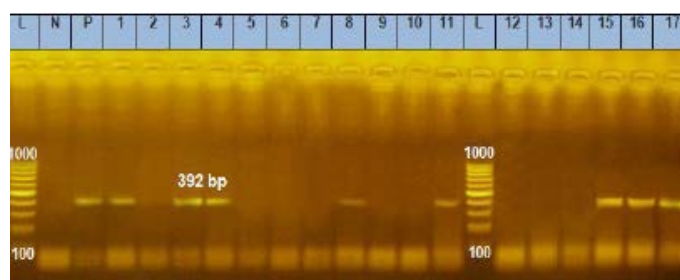
**Figure 1:** PCR for *P. aeruginosa* 16S rDNA (956bp). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive *P. aeruginosa*. Lane N: Control negative. Lanes from 1 to 17: Positive *P. aeruginosa* 16S rDNA (956bp).



**Figure 2:** PCR for *mexR* gene at (637pbb). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive. Lane N: Control negative. Lanes 1 to 6, 8, 13, 16, 17: Positive for *mexR* (637bp).



**Figure 3:** PCR for *bla*TEM gene at (516bp). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive *P. aeruginosa*. Lane N: Control negative. Lanes 1, 2, 4 to 8, 10, 13, 16, 17: Positive for *bla*TEM gene (516pb).



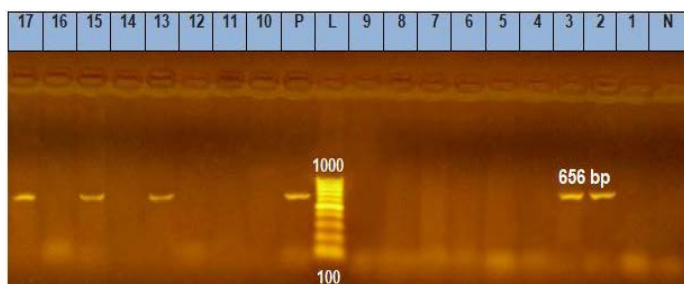
**Figure 4:** PCR for *bla*SHV gene at (392bp). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive. Lane N: Control negative. Lanes 1, 3, 4, 8, 11, 15, 16, 17: Positive for *bla*SHV gene (392bp).



**Figure 5:** PCR for *bla*CTX-M gene at (593bp). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive. Lane N: Control negative. Lanes 1, 2, 5, 15, 17: Positive for *bla*CTX-M gene (593pb).

The dromedary camel is a good source of meat and milk in semiarid and arid zones. This is because of the unique

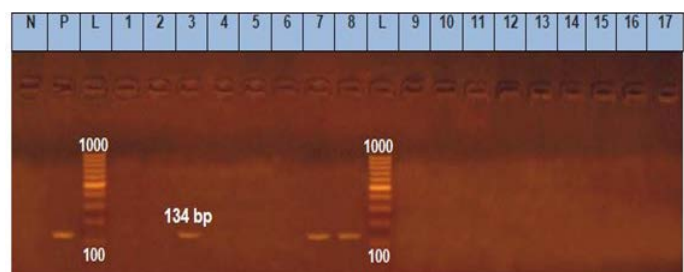
physiological characteristics of camels (Kadim et al., 2008). Therefore, as they are important food sources, the camel industry is in transition from nomadism to intensive production (FAO, 2019).



**Figure 6:** PCR for *pslA* gene at (656bp). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive. Lane N: Control negative. Lanes 2, 3, 13, 15, 17: Positive for *pslA* gene (656bp).



**Figure 7:** PCR for *toxA* gene at (396bp). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive. Lane N: Control negative. Lanes 3, 10, 13, 16: Positive for *toxA* gene (396bp).



**Figure 8:** PCR for *exoU* gene at (134bp). Lane L: 100-1000 bp ladder molecular size (DNA marker). Lane P: Control positive. Lane N: Control negative. Lanes: 3, 7, 8: Positive for *exoU* gene (134bp).

*Pseudomonas aeruginosa* is one of the major causes of diseases in camel such as hemorrhagic pneumonia, otitis, mastitis, endometritis, and urinary tract infections (Salomonsen, 2013). In this study, the prevalence of *P. aeruginosa* in camels was determined about 12.0% (30/250). This percent is so nearly to the encountered investigations from camel respiratory tract in Egypt 11.0% which considered as one of the most common opportunistic Gram-negative bacteria (Ismail et al., 2014). *Pseudomonas aeruginosa* and other gram-negative bacteria with ESBLs contain other

$\beta$ -lactamases that makes difficult the phenotypic detection of ESBL (Manchanda and Singh, 2003). All of ruminants depend on eructation, which directly reflected on the microflora in the nasal passages and that explain the main reason for highest percent of bacterial isolation form nasal swabs either in diseased or apparent healthy camels that shown in Table 2. The authors attributed the little increase in result to that, most of samples are collected from animals at regions of pre slaughtering or after transportation, which the animals may subjected to various stress and predisposing factors could augment *P. aeruginosa* growth and increase it recovery rate from respiratory passages. *P. aeruginosa* which considered as one of the most common opportunistic Gram-negative bacteria (Tavajjohi et al., 2011) In this point, there is a considerable report for potential transfer of *P. aeruginosa* in between animal and humans.

Food-producing animals may be an important vehicle for the community wide dissemination of antimicrobial resistant *Enterobacteriaceae* and *P. aeruginosa* especially ESBL-producing type isolates have been found in increasing numbers in food-producing animals (Zurfluh et al., 2016).

ESBL-producing bacteria are one of the fastest emerging resistance problems worldwide. ESBL-producing bacteria were observed in human medical practice, the observation of these bacteria in companion animals and the increase in livestock has initiated monitoring studies concentrating on livestock (Ewers et al., 2011). Accordingly to the hypothesis that animals might become infection sources or even natural persistent sources acting as risky reservoirs of infection leading to the spread of these bacteria specifically multidrug resistant types in community (Watkins and Bonomo, 2016). Molecular identification clearly indicated the presence of virulence gene among studied isolates. *pslA* was present in 29.4% of examined isolates, while *toxA* 23.5% and *exoU* 17.6% as shown in Table 4 the *toxA* gene represented in percentage (35.29%) in Fazeli and Momtaz (2014) while (Azimi et al., 2016) mentioned that 52% of the isolates carried *exoU*, and 26.3% carried *exoS*.

ESBLs are typically identified in *P. aeruginosa* isolates and showing resistance to the extended-spectrum cephalosporin (ESCs) (Fadlelmula et al., 2016), this resistance is often due to the production of  $\beta$ -lactamases. Clinically, ESBLs are generally encoded by plasmid-mediated *bla* genes; three major clinically relevant  $\beta$ -lactamase genes are *blaSHV*, *blaTEM* and *blaCTX-M* (Bush, 2013). The total percentage of ESBL producing *P. aeruginosa* was 56.6% (17/30) from camel samples by DDST, accordingly, the most frequently  $\beta$ -lactamase-genes detected in this isolate by PCR using specific primers were *blaTEM* (64.7%) followed by *blaSHV* (47.0%) and *blaCTX-M* (29.4%).



The ESBL encoding genes also detected from camels' meat samples in Egypt as follow *bla*CTX-M (38%), followed by *bla*SHV (33.3%) and *bla*TEM, *bla*PER-1 (28.5%) (Elhariri et al., 2017) this indicates the variation of ESBL encoding genes from different isolates which prove that *P. aeruginosa* and other gram-negative bacteria with ESBLs contain other  $\beta$ -lactamases that makes difficult the phenotypic detection of ESBL (Chander and Raza, 2013) this issue need further investigation so, in this study by using PCR for identify *mexR* gene which present in percentage 58.8% (10/17) from *P. aeruginosa* isolates. Bacterial multidrug efflux pumps play an important role in the antimicrobial resistance of gram-negative pathogens (Poole, 2001). In the present study Antibiotic resistance pattern of ESBL producing *P. aeruginosa* showed high-level resistance (100%) to 3<sup>rd</sup> generation cephalosporine cefotaxime and 4<sup>th</sup> generation cephalosporine cefepime followed by meropenem (88.2%) and imipenem (82.4%) and Penicillin G, Gentamicin and aztreonam (82.3%, 76.4% and 70.5%), respectively with high sensitive for ofloxacin (100%) followed by Sulphamethoxazole/ trimethoprim and erythromycin (70.5%) as shown in Table 4. This pattern are nearly similar to ESBL *P. aeruginosa* which high level resistance (100%) to ceftazidime, ceftriaxone and rifampicin followed by cefepime (95.2%) and aztreonam (76.1%) (Elhariri et al., 2017) and *P. aeruginosa* are multi-drug resistant to amikacin (17.25%), ciprofloxacin (27.59%), ceftriaxone varied from 51.0 to 73.0% and all the strains were susceptible to imipenem (20.69%) (Chander and Raza, 2013). So, the presence of high resistance profile by camel *P. aeruginosa* isolates my attributed antibiotics used in management of this animals or natural resistance of camel that suites it as a risk reservoir for such pathogens.

## CONCLUSIONS AND RECOMMENDATIONS

*P. aeruginosa* is an important incriminated pathogen in camel. Increasing resistance to beta-lactams in *P. aeruginosa* has become a serious threat, particularly against third and fourth generation cephalosporins. There are a lot of molecular mechanisms to develop resistance against these antibiotics; generation of extended-spectrum beta-lactamases (ESBL), by incorporation of *bla* genes in integrons and inability of porin genes to enhance their expression level and/or alteration of antibiotic target sites.

## NOVELTY STATEMENT

As shortage of papers on camel diseases, this paper shed light on the *Pseudomonas* and their importance in camels as has become a serious threat as well as detection of their virulence genes particularly resistance development against 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins.

All authors share in the work design, practical section as well as, analysis of the results, writing and revising of the manuscript.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## REFERENCES

- Archambault M, Petrov P, Hendriksen RS, Asseva G, Bangtrakulnonth A, Hasman H, Aarestrup FM (2006). Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among *Salmonella enterica* serovar Corvallis from Thailand, Bulgaria, and Denmark. *Microb. Drug Resist.* 2006 Fall;12(3):192-8.
- Azimi S, Kafil HS, Baghi HB, Shokrian S, Najaf K, Asgharzadeh M, Aghazadeh M (2016). Presence of *exoY*, *exoS*, *exoU* and *exoT* genes, antibiotic resistance and biofilm production among *Pseudomonas aeruginosa* isolates in Northwest Iran. *GMS hygiene and infection control*, pp. 11.
- Bauer AW, Kirby WM, Sherris JC, Antibiotic MT (1966). susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45(4): 493-496. [https://doi.org/10.1093/ajcp/45.4\\_ts.493](https://doi.org/10.1093/ajcp/45.4_ts.493)
- BioMe'rieux (2006). Vitek2 product information, document 510769-4EN1. biomerieux, Inc., Durham, NC.
- Breidenstein EB, de la Fuente-Núñez C, Hancock RE (2011). *Pseudomonas aeruginosa*: All roads lead to resistance. *Trends Microbiol.*, 19(8): 419-426. <https://doi.org/10.1016/j.tim.2011.04.005>
- Bush K (2013). The ABCD's of  $\beta$ -lactamase nomenclature. *J. Infect. Chemother.*, 19(4): 549-559. <https://doi.org/10.1007/s10156-013-0640-7>
- Chander A, Raza MS (2013). Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* clinical isolates at a tertiary care hospital in Kathmandu, Nepal (2013). *Asian J. Pharm. Clin. Res.*, 6(3): 235-238.
- CheeSanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC, Maxwell S, Aminov RI (2009). Fate and transport of antibiotic residues and antibiotic resistance genes. *J. Environ. Qual.*, 38(3): 1086-1108. <https://doi.org/10.2134/jeq2008.0128>
- CLSI, 2020. Performance standards for antimicrobial susceptibility testing. 30<sup>th</sup> edition. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
- Colom K, PérezJ, Alonso R, Fernández-AranguizA, LariñoE, Cisterna R (2003). Simple and reliable multiplex PCR assay for detection of *bla*TEM, *bla*SHV and *bla*OXA-1 genes in Enterobacteriaceae. *FEMS Microbiol. Lett.* 223 (2003) 147-151.
- Elhariri M, Dalia H, Rehab E, Sohad MD (2017). Extended spectrum beta lactamase producing *Pseudomonas aeruginosa* in camel in Egypt: potential human hazard. *Ann. Clin. Microbiol. Antimicrob.*, 16: 21. <https://doi.org/10.1186/s12941-017-0197-x>
- Ewers C, Grobbel M, Bethe A, Wieler LH, Guenther S (2011). Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: Action is clearly warranted. *Berl. Munch. Tierarztl. Wochenschr.*, 124(3/4):

- 4–101.
- Fadlilmula A, Al-Hamam NA, Al-Dughaym AM (2016). A potential camel reservoir for extended spectrum  $\beta$ -lactamase-producing *Escherichia coli* causing human infection in Saudi Arabia. *Trop. Anim. Health Prod.*, 48(2): 427–433. <https://doi.org/10.1007/s11250-015-0970-9>
- FAO (2019). Food and Agriculture organization of the United Nations statics division. <http://www.fao.org/fastat>. Accessed 27 Oct 2019.
- Fazeli N, Momtaz H (2014). Virulence gene profiles of multidrug-resistant *Pseudomonas aeruginosa* isolated from Iranian hospital infections. *Iran. Red Crescent Med. J.*, 16(10): e15722. <https://doi.org/10.5812/ircmj.15722>
- Ghadaksaz A, Fooladi AAA, Hosseini HH, Amin M (2015). The prevalence of some *Pseudomonas* virulence genes related to biofilm formation and alginate production among clinical isolates. *J. Appl. Biomed.* 13(1): 61–68.
- Ismail M, El-Deen NE, El-Hariri (2014). M. Bacteriological examination of respiratory tract of apparently healthy camels in Egypt. *Int. J.*, 5(1): 65–68.
- Jarlier V, Nicolas MH, Fournier G, Philippon (1988). A. Extended spectrum  $\beta$ -lactamases conferring transferable resistance to newer  $\beta$ -lactam agents in enterobacteriaceae: Hospital prevalence and susceptibility patterns. *Rev. Infect. Dis.*, 10: 867–878. <https://doi.org/10.1093/clinids/10.4.867>
- Kadim IT, Mahgoub O, Purchas RW (2008). A review of the growth, and of the carcass and meat quality characteristics of the one-humped camel (*Camelus dromedaries*). *Meat Sci.*, 80(3): 555–569. <https://doi.org/10.1016/j.meatsci.2008.02.010>
- Matar GM, Ramlawi F, Hijazi N, Khneisser I, Abdelnoor AM (2002). Transcription Levels of *Pseudomonas aeruginosa* Exotoxin A Gene and Severity of Symptoms in Patients with Otitis Externa. *Curr. Microbiol.* 45: 350–354.
- Manchanda V, Singh NP (2003). Occurrence and detection of AmpC beta-lactamases among gram negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. *J. Antimicrob. Chemother.*, 51: 415–418. <https://doi.org/10.1093/jac/dkg098>
- Poole K (2001). Multidrug resistance in gram-negative bacteria. *Curr. Opin. Microbiol.*, 4: 500–508. [https://doi.org/10.1016/S1369-5274\(00\)00242-3](https://doi.org/10.1016/S1369-5274(00)00242-3)
- Quinn PJ, Carter ME, Markey BK, Carter GR (2004). *Veterinary clinical microbiology*, wolfe publication, London, UK, pp. 254–258.
- Sahar RM, Ibrahim EM, El-Bardisy MM, Aziz HM, Amany ND, Hala FH (2014). Evaluation of the automated VITEK2 compact system in the identification of Gram Positive Cocci in comparison to the conventional identification methods. *Anim. Health Res. J.*, 2(4): 37– 48.
- Salomonsen CM, Themudo GE, Jelsbak L, Molin S, Høiby N, Hammer AS (2013). Typing of *Pseudomonas aeruginosa* from hemorrhagic pneumonia in mink (*Neovison vison*). *Vet. Microbiol.*, 163(1): 103–109. <https://doi.org/10.1016/j.vetmic.2012.12.003>
- Sánchez P, Linares JF, Ruiz-Díez B, Campanario S, Navas A, Baquero F, Martínez JL (2002). Fitness of in vitro selected *Pseudomonas aeruginosa* nalB and nfxB multidrug resistant mutants. *J. Antimicrob. Chemotherap.* 50: 657–664.
- Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Herman L, Haesebrouck F, Butaye P (2010). Broad-spectrum  $\beta$ -lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. *FEMS Microbiol. Rev.*, 34(3): 295–316. <https://doi.org/10.1111/j.1574-6976.2009.00198.x>
- Spilker T, Coenye T, Vandamme P, LiPuma JJ (2004). PCR-Based Assay for Differentiation of *Pseudomonas aeruginosa* from Other *Pseudomonas* Species Recovered from Cystic Fibrosis Patients. *J. Clin. Microbiol.* 2074–2079.
- Strauß LM, Dahms C, Becker K, Kramer A, Kaase M, Mellmann A (2015). Development and evaluation of a novel universal  $\beta$ -lactamase gene subtyping assay for *blaSHV*, *blaTEM* and *blaCTX-M* using clinical and livestock-associated *Escherichia coli*. *J. Antimicrob. Chemother.*, 70(3): 710–715. <https://doi.org/10.1093/jac/dku450>
- Tavajjohi Z, Moniri R, Khorshidi A (2011). Detection and characterization of multidrug resistance and extended-spectrum-beta-lactamase-producing (ESBLs) *Pseudomonas aeruginosa* isolates in teaching hospital. *Afr. J. Micro-Biol. Res.*, 5(20): 3223–3228. <https://doi.org/10.5897/AJMR11.260>
- Watkins RR, Bonomo RA (2016). Global and local impact of antibiotic resistance. *Infect. Dis. Clin. North Am.*, 30(2): 313–322. <https://doi.org/10.1016/j.idc.2016.02.001>
- Winstanley C, Kaye SB, Neal TJ, Chilton HJ, Miksch S, Hart CA. and the Microbiology Ophthalmic Group (2005). Genotypic and phenotypic characteristics of *Pseudomonas aeruginosa* isolates associated with ulcerative keratitis. *J. Med. Microbiol.* 54: 519–526.
- Zurfluh K, Hindermann D, Nüesch-Inderbinen M, Poirer L, Nordmann P, Stephan R (2016). Occurrence and features of chromosomally encoded carbapenemases in gram-negative bacteria in farm animals sampled at slaughterhouse level. *Schweiz Arch Tierheilkd.*, 158(6): 457–460. <https://doi.org/10.17236/sat00072>