REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

# Phenotypic and genotypic characterization of Extended Spectrum β-lactamases producing *Proteus mirabilis* isolates.

**Lamiaa A. Salama[d], Hazem Hamed Saleh[b], Shaymaa H. Abdel-Rhman[a,c], Rasha Barwa[a]*, Ramadan Hassan[a]**

**[a] Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.**
**[b] Urology and Nephrology center, Mansoura University.**
**[c] Department of Pharmaceutics and Pharmaceutical Biotechnology, Faculty of Pharmacy, Taibah University, AL Madinah Al Munawara, Kingdom of Saudi Arabia.**
**[d] Microbiology and Immunology Department, Faculty of pharmacy, Horus university, New Damietta 34518, Egypt.**

**Received on: 28. 06. 2021**
**Revised on: 01. 07. 2021**
**Accepted on: 04. 07. 2021**

***Corresponding Author:***

Rasha Barwa.

Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Egypt.

**E. mail**: rasha2000@gmail.com rasha@mans.edu.eg

**Fax**: (+2) 050-2246253

**Phone**: (+2) 01001964899

**Mail Address:**
Microbiology & Immunology Department, Faculty of Pharmacy, Mansoura University. Mansoura 35516, Egypt.

## ABSTRACT

**Background:** The Extended spectrum β-lactamases (ESBLs) production is one of the most essential mechanism of drug resistance in *Proteus mirabilis* (*P. mirabilis*). **Objective:** Phenotypic and genotypic detection of extended spectrum β -lactamases production in *P. mirabilis* and its antimicrobial resistance among clinical isolates. **Methods:** A total of Sixty *P. mirabilis* clinical isolates were tested for Extended spectrum β lactamase production by modified double disc synergy test (MDDST). Polymerase chain reaction (PCR) was done to detect Extended spectrum β-lactamases genes in the clinical isolates. Antimicrobial susceptibility testing was done by means of Kirby Bauer disc diffusion method. **Results:** Out of the 60 *P. mirabilis* clinical isolates, 17 isolates (28.33%) were ESBL producers by phenotypic test. Genotypically; ESBLs genes were detected and the most prevalent ESBL resistance gene was TEM (91.7%) followed by SHV (75%), CTX-2-59 (56.7%) and CtxM-15 (51.7%). Moderate level of resistance to ofloxacin, ciprofloxacin, amoxicillin/ clavulanic acid, cefotaxime and ceftazidime (60.0%, 58.3%, 45.0%, 41.7% and 40.0% respectively) was recorded. In addition, 73.33% of isolates were classified as multidrug resistant (MDR). **Conclusion:** Monitoring of Extended spectrum β-lactamase producing *P. mirabilis* is very important because of its high prevalence among urinary tract infections. Also; increasing awareness for clinicians and enhancing laboratories tests leading to reduce the spread of these isolates.

***Keywords :P. miribilis, Extended spectrum β-lactamase, antimicrobial resistance, urinary tract infections.***

## INTRODUCTION

*Proteus mirabilis* (*P. mirabilis*) is a Gram-negative rod-shaped bacterium frequently noted for its swarming motility and urease activity. It considers as an important uropathogen between patients with complicated urinary tract, urolithiasis and extended-term urinary catheterization (CAUTI). These infections might be developed to renal stones due to alkalization of urine. Presence of ammonia resulted from urease-catalyzed urea hydrolysis leading to magnesium and calcium crystallization which could obstruct the lumen of indwelling catheters (Ali and Yousif, 2015, Armbruster *et al.*, 2018).

Cephalosporins and β-lactam/β-lactamase inhibitors were found to be effective against *P. mirabilis*. Though, strains resistant to β-lactams intermediated by developed β-lactamases appeared in 1990s. Plasmid borne extended-spectrum β-lactamases (ESBLs) were the greatest worrisome among these β-lactamases due to their resistance to almost penicillins and cephalosporins and their ability to spread amongst several species of *Enterobacteriaceae*. Numerous reports had represented that ESBLs β-lactamase- *P. mirabilis* producer isolates could initiate clonal widespread. Thus, wide nosocomial outbreaks and community acquired infections were developed. Failure of treatment and clinical morbidity were also extra predictable to happen in patients infected by ESBLs-producing *P. mirabilis*, which had been estimated to inadequate therapy (Wang *et al.*, 2014). Mostly ESBLs were derivatives of *TEM-1* type, *TEM-2* type and *SHV-1* type of β-lactamase, that were confined one point code gene mutations or more. Recently, Increasing studies of *CTX-M*-type ESBLs were developed by *Proteus mirabilis* as well as *TEM*-type ESBLs (Huang *et al.*, 2014).

World Health Organization (WHO) reported that the most serious problem was the routine use of antibiotics leading to the incidence of antimicrobial drug resistant and development of antimicrobial resistant genes. *P. mirabilis* has been concerned in several nosocomial infection outbreaks and community acquired infections in different parts of the world. The most predominant classes of antibiotics prescribed for life threatening cases are the 3rd generation cephalosporins, fluoroquinolones and aminoglycosides. Finally, several studies have reported high resistance of *Enterobacteriaceae* including *P. mirabilis* to these classes of antibiotics (Pawar *et al.*, 2018). MDR (Multidrug resistance) recognized as developed resistance to at least one antimicrobial category agent, XDR (extremely drug-resistant) recognized as resistance to not less than one or two antimicrobial agents (bacterial isolates susceptible to at least two categories) and PDR (pan drug-resistant bacteria) recognized as resistance to all antimicrobial agents in all antimicrobial categories (Magiorakos *et al.*, 2012). In our study to ensure MDR definition, clinical isolates were tested against different antimicrobial agents with variant antimicrobial categories.

Current study aimed to explore the genotype of ESBLs of *P. mirabilis* clinical isolates isolated from urinary tract infection from Urology and Nephrology center, Mansoura University Hospitals, Dakahlia governorate, Egypt, with recognition of their antibiotic resistance.

## MATERIALS AND METHODS

### Organisms:

- **Bacterial isolates:**

During the period from October 2016 to April 2017, 300 clinical specimens were collected from Urology and Nephrology center (UNC), Mansoura University Hospitals, Dakahlia governorate, Egypt. All isolates were checked and identified using automated VITEK-2 system (bioMerieux, Marcy I'Etoile, France). The research proposal was approved from research ethics committee of faculty of pharmacy, Mansoura University.

- **Standard strains:**

For quality control; *Escherichia coli* (ATCC-25922), *Klebsiella pneumoniae* (ATCC-700603), *Klebsiella pneumoniae* (ATCC BAA-1705) and *Klebsiella pneumoniae* (ATCC BAA-1706) were provided by UNC.

### Determination of antimicrobial susceptibility pattern of *P. mirabilis* isolates:

Susceptibility to different antimicrobials was determined by Kirby-Bauer disc diffusion technique according to the clinical laboratory standard institute (**CLSI**, **2017**). Eleven antimicrobial discs of various categories were used including; amikacin 30 µg (aminoglycosides), amoxicillin/clavulanic acid 20/10 µg (penicillin/ β-lactamase inhibitor), ciprofloxacin 5 µg, ofloxacin 5 µg (second generation fluoroquinolone), ceftazidime 30 µg and cefotaxime 30 µg (extended spectrum cephalosporins), imipenem 10 µg and etrapenem 10 µg (carbapenem),

pipracillin/tazobactam 100/10 µg (anti pseudomonal penicillin/ β-lactamase inhibitor), Trimethoprim/Sulphamethoxazole 25 µg (folate pathway inhibitor), and nitrofurantoin 300 µg (antiseptic drug); all discs were supplied from Bioanalyze ® products, Turkey. In this study, MDR was evaluated as resistance to at least one agent in three or more antimicrobial categories (Tambekar *et al.*, 2006, Magiorakos *et al.*, 2012).

**Phenotypic Extended Spectrum β-lactamases (ESBLs) detection using modified double disc synergy test (MDDST):**

Screening of ESBLs-producers was done by determination of minimum inhibitory concentration (MIC) for ceftazidime and cefotaxime. If MIC of ceftazidime and cefotaxime was ≥ 2 µgl/ ml, the isolate is considered ESBL-producer (CLSI, 2017).

MDDST was applied to confirm the ESBLs production among *P. mirabilis* clinical isolates by using a disc of amoxicillin-clavulanate (20/10 µg) along with ceftazidime (30 µg) and cefotaxime (30 µg). *Escherichia coli* (ATCC-25922) was used as a negative control and *Klebsiella pneumoniae* (ATCC-700603) was used as a positive control for the ESBL production (Kaur *et al.*, 2013).

**Detection of Extended Spectrum β-lactamase genes using PCR:**

The nucleotide sequence, product size and annealing temperature of the primers used in detection of resistance genes illustrated in table (1). The PCR amplification was performed in a total volume of 25 µl containing 12.5 µl of DreamTaq™ Green PCR master mix, 1 µl DNA extract, 1µl of forward primer (10 µM), 1 µl of reverse primer (10 µM) and 9.5 µl of nuclease free water. PCR was performed in Master cycler epgradient S thermacycler (Epindorf, Mississauga, ON, Canada) as well as a negative control reaction with each primer. The PCR reactions for each gene was performed according to the cycling conditions listed in the following table (2).

## STATISTICAL ANALYSIS

To collect descriptive results, data were tabulated, coded, and evaluated using the computer software SPSS version 26.0. The following descriptive statistics were calculated: Interquartile distribution and median (IQR) and Recurrence (Number-percent).

Analytical statistics: The magnitude of variance was checked using one of the following tests in the statistical comparison between the various groups: Mann Whitney, Pearson's chi square test ($X^2$-value) or fisher exact, and Monte-Carlo. Spearman's correlation coefficient test was used correlating different parameters. A P value <0.05 was considered statistically significant.

**Table (1): The primer sequences of Extended Spectrum β-lactamase gene detection of 60 *P. mirabilis* clinical isolates (Gharrah *et al.*, 2017).**

| Target gene | Type | Nucleotide sequence (5` to 3`) | Product size | Annealing temperature |
|---|---|---|---|---|
| *Ctx M-15* | F | GTGATACCACTTCACCTC | 255 | 54° C |
| | R | AGTAAGTGACCAGAATCAG | | |
| *CTX-2-59* | F | GATGACTCAGAGCATTCG | 739 | 54°C |
| | R | GTTGGTGGTGCCATAATC | | |
| *SHV* | F | ACTATCGCCAGCAGGATC | 356 | 58°C |
| | R | ATCGTCCACCATCCACTG | | |
| *TEM* | F | GATCTCAACAGCGGTAAG | 786 | 54°C |
| | R | CAGTGAGGCACCTATCTC | | |

**Table (2): The primer cycling conditions for PCR reactions of 60 *P. mirabilis* isolates.**

| Target gene | One cycle | Cycling condition | | | | One cycle |
|---|---|---|---|---|---|---|
| | Initial Denaturation | Denaturation | Annealing | Extension | No. of cycles | Final Extension |
| Ctx M-15 | 95°C/ 5min | 95°C/30 sec | 54° C/ 30sec | 72°C/ 1min | 35 cycles | 72°C/ 5min |
| CTX-2-59 | | | 54°C/ 30sec | | | |
| SHV | | | 58°C/ 30sec | | | |
| TEM | | | 54°C/ 30sec | | | |

## RESULTS

### Collection, isolation and identification of clinical isolates:

Out of 300 clinical isolates; 60 isolates of *Proteus mirabilis* were identified using automated VITEK-2 system (bioMerieux, Marcy I'Etoile, France). The most prevalent diagnosis of isolated samples was renal stone cases as it represented 16.70% followed by hypospadias cases (13.30%), then post repair of vesicocutaneous fistula (8.33%). The common of cases represented by males (72%).

### Determination of antimicrobial susceptibility pattern of all *P. mirabilis* isolates:

The antimicrobial susceptibility patterns of *P. mirabilis* isolates were evaluated by the disc-diffusion method. The lowest level of resistance was observed with amikacin, imipenem, pipracillin/ tazobactam and etrapenem (8.3%, 11.7%, 11.7% and 13.3% respectively). Moderate level of resistance to ofloxacin, ciprofloxacin, amoxicillin/ clavulanic acid, cefotaxime and ceftazidime (60.0%, 58.3%, 45.0%, 41.7% and 40.0% respectively) was observed. The maximum level of resistance was recorded by nitrofurantoin (98.3%) and trimethoprim/ sulfamethoxazole (81.7%). Moreover, 44 (73.33%) isolates were MDR; distributed with the highest percentage in the median age.

### Phenotypic detection of Extended Spectrum β- lactamases (ESBLs) by modified double disc synergy test:

Seventeen isolates (28.33%) were found to have MIC of ceftazidime and cefotaxime ≥ 2 µgl/ ml, so they were considered as ESBL-producers and these results were confirmed by MDDST test. The third generation cephalosporins represented the highest percent for resistance among different isolates. By comparing its resistance statistically, 3rd generation cephalosporins is more prevalent than penicillins group (P-value=0.04) and higher than carbapenems group (P-value≤ 0.001). Moreover, penicillins group represented higher percent for resistance than carbapenems among different isolates (P-value=0.002) as illustrated in figure (1).

### PCR detection of resistance genes:

Different Extended spectrum beta-lactamase genes (*CtxM-15, CTX-2-59, SHV, TEM*) were detected by PCR. It was found that the most prevalent resistance gene was *TEM* (91.7%) followed by *SHV* (75%), *CTX-2-59* (56.7%) and *CtxM-15* (51.7%) shown in fig. (2).

The relation between phenotypic ESBL-producer and genotypic ESBL resistance genes represented in fig. (3); Although 17 (28.3%) isolates gave positive ESBLs producer by phenotypic test and 43 (71.7%) negative ESBL-producer isolates, but also had verified results against selected ESBL-resistance genes. It has shown no significance P-value.

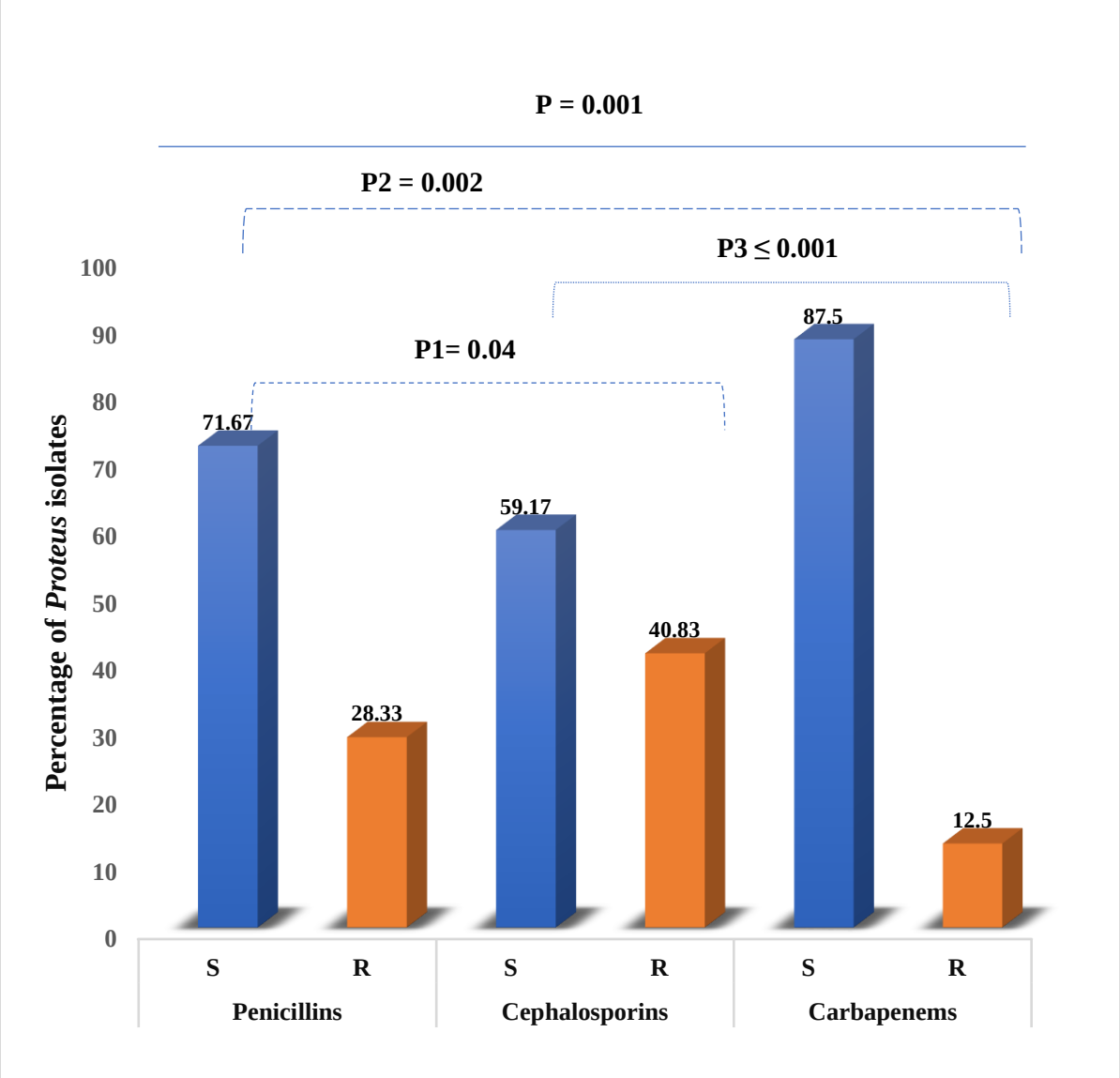



**Fig. (1): Resistance percentage of β-lactam classes among different *P. mirabilis* clinical isolates.**

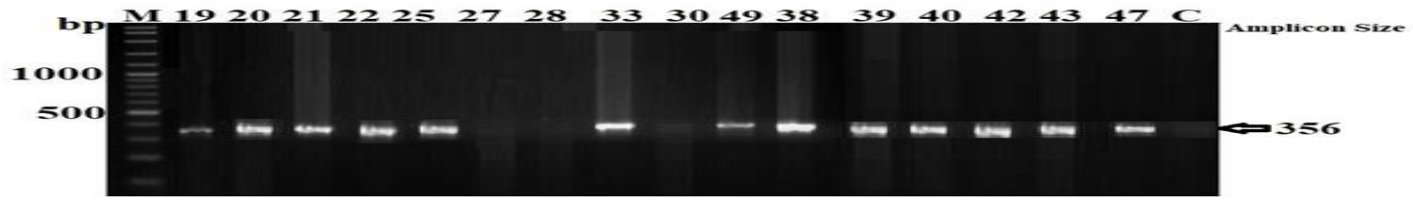
***: (P) Probability between all β-lactam classes.***

***: (P1) Probability between Pencillins & Cephalosporins.***

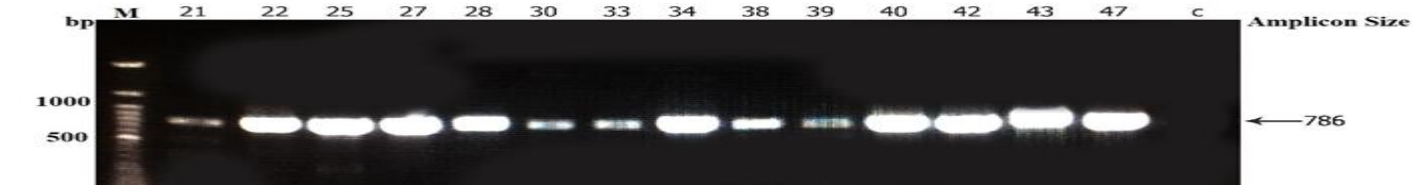
***: (P2) Probability between Pencillins & Carbapenems.***

***: (P3) Probability between Cephalosporins & Carbapenems.***

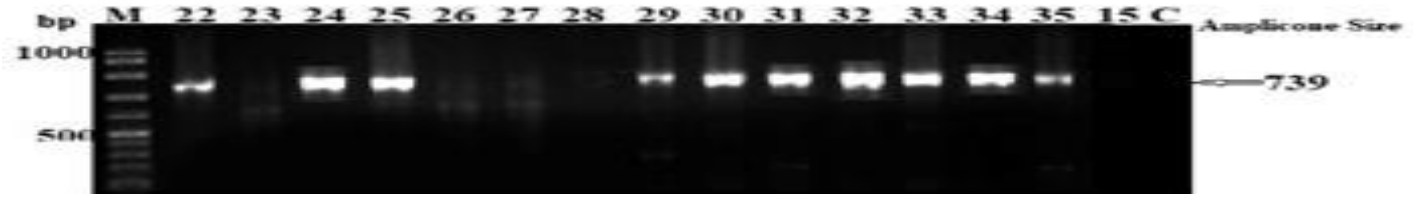
**A)**




**B)**




**C)**




**D)**

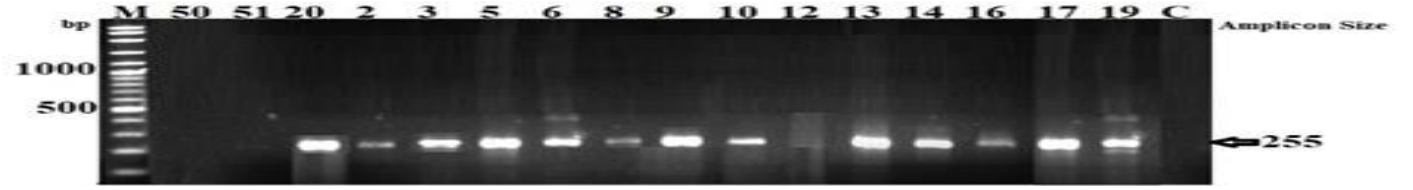



**Fig. (2): Agarose gel electrophoresis of different ESBLs genes among *Proteus* isolates. Lane C: negative control (without DNA template), bp: base pair.**
**A) *SHV*. B) *TEM*. C) *CTX-2-59*. D) *CtxM-15*.**

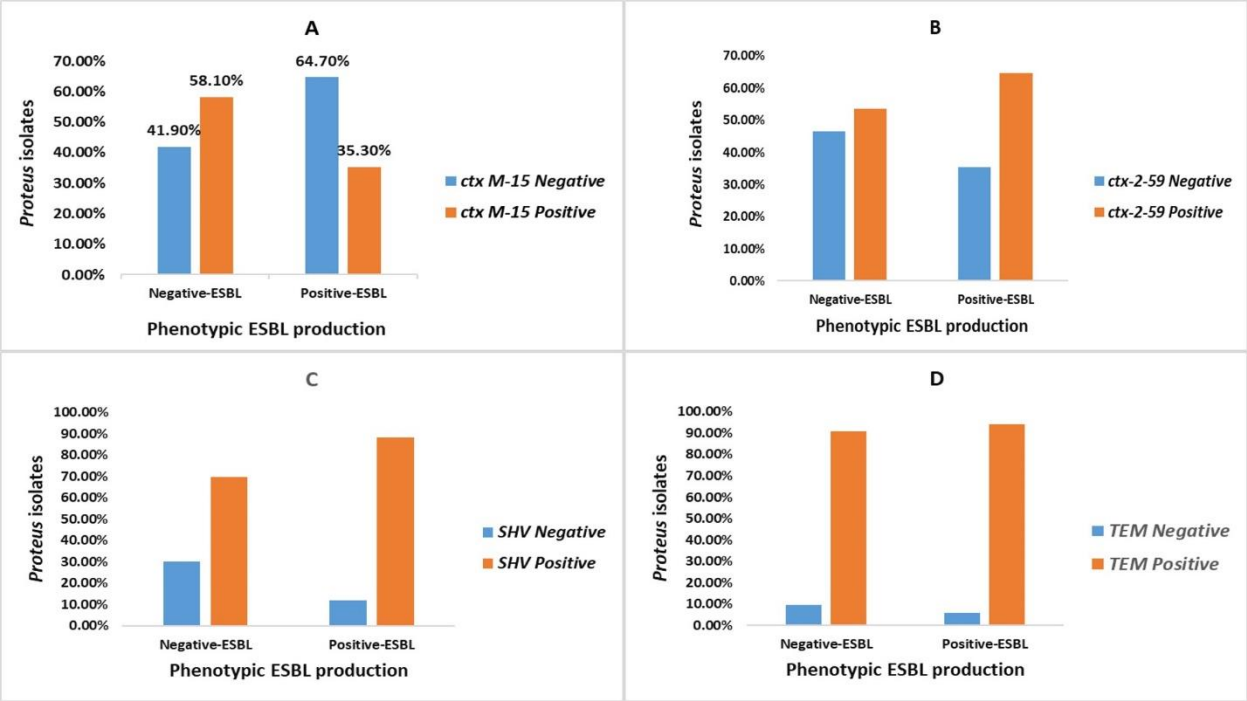



**Fig. (3): Distribution of ESBLs genes among positive and negative *Proteus* isolates producers.**

**A) *CtxM-15.*** **B) *CTX-2-59.*** **C) *SHV.*** **D) *TEM.***

*Proteus mirabilis* is a Gram-negative bacterium which has been notorious for its ability to swarm firmly across surfaces in a striking bull's eye pattern. Clinically, it exhibits an extraordinary lifestyle as uropathogen invading human urinary tracts. *P. mirabilis* considered as the 2nd greatest predominant uropathogen about 5.2% in the male patients using indwelling catheter (Gravey *et al.*, 2017). It considers as a model microorganism for urease-producing pathogens on indwelling urinary catheters, often leading to poly-microbial infection. Recent studies have illustrated how *P. mirabilis* causes all of these diseases.

In our study; It was found that 44 (73.33%) isolates were MDR, distributed with the highest percentage in the median age. 3rd generation cephalosporins is more prevalent than penicillins group (P-value=0.04) and higher than carbapenems group (P-value ≤ 0.001).

Moreover, penicillins group represented higher percent for resistance than carbapenems among different isolates (P- value=0.002).

(Korytny *et al.*, 2016) reported similar results where 55.6% were MDR.

Resemble results of (Danilo de Oliveira *et al.*, 2021); showing that the highest resistance rate was found to trimethoprim/ sulfamethoxazole combination. A study performed by (Gravey *et al.*, 2017) showed different resistance rates for gentamicin (18%), phosphomycin (19%), ciprofloxacin (21%), norfloxacin (22%), nalidixic acid (26%), trimethoprim/ sulfamethoxazole (32%), ampicillin and amoxicillin (40%), and ticarcillin (42%) (Gravey *et al.*, 2017).

In recent years, the infectious diseases treatment has been a significant matter for human security and the contineous increased bacterial resistance elevated patients' expenditures. The ESBLs production has considered as the major hazard due to the usage of the novel generation of cephalosporins.

*Enterobacteriaceae* rate of production of ESBLs has been significantly elevated (Jain and Mondal, 2008, Ishikawa *et al.*, 2011).

Among *Enterobacteriaceae*, *K. pneumoniae, Escherichia coli* and *Proteus mirabilis* were almost vital causative agents of community-acquired infections (Karimisup *et al.*, 2012). Prevelance of infection achieved by ESBLs producing *Enterobacteriaceae* lead to broad use of the cephalosporins generations (Gholipour *et al.*, 2014).

In our study, seventeen (28.3%) *P. mirabilis* clinical isolates gave positive ESBL producer by phenotypic test. In Japans hospitals, the ESBL prevalence producing strains in *P. mirabilis* varies about 11.9 to 37.8%, depending on the locations and studied reports (Kanayama et al., 2010, Ishikawa et al., 2011). Another study; found that 20% of the prevalence of ESBL-producing *Proteus* has been around, which is similar to prior results (Kurihara *et al.*, 2013).

In our study; The most predominant ESBL resistance gene was *TEM* (91.7%) followed by *SHV* (75%), *CTX-2-59* (56.7%) and *CtxM-15* (51.7%). Another study showed that the most predominant type of ESBLs enzymes were the *bla CTX M* type β-lactamases accounting for about 95% of most types of ESBL-enzymes practically (Gajdács and Urbán, 2019).

From (2000 to 2004), *P. mirabilis* ESBLs producer isolates had been increased gradually in Japan to a highly dramaturgical level representing 46.2% (Nakano *et al.*, 2012) more elevated than France representing about 6.9% (Chanal *et al.*, 2000) while in United States representing about 9.5% (Saurina *et al.*, 2000). Recently; Nakama study performed from 2013 and 2014 in Japanese hospital and had reported that the prevalence of *P. mirabilis* producing *CTX M2* and *CTX M14* were about 11.1% of the *Proteus* isolates against 11.5% of *E. coli* isolates, and *K. pneumoniae* isolates about 6.2% (Nakama *et al.*, 2016). In Argentina, *CTX M2* ESBLs had represented in *P. mirabilis* (Quinteros *et al.*, 2003), Spain (Mata *et al.*, 2011) and Italy (Pagani *et al.*, 2003). Most *β*-lactamases donate a dissimilarity resistance to antibiotics involving the 3$^{rd}$ and 4$^{th}$ generation cephalosporins. (Gharrah *et al.*, 2017) confirm that ESBLs producing isolates revealed significantly superior resistance to the tested β-lactamase than non-ESBLs producers ($P$ < 0.0001).

Those results were compared with the previous studies by (Shin *et al.*, 2014), wherever ESBLs producing isolates represnted advanced significant resistant to almost β lactamase non-ESBLs producing isolates ($P$ < 0.05).

Several studies showed that the rate of ESBL-producing organisms was significantly higher among in-patients hospitalized comparing to out-patients (p < 0.001). This pattern could be explained by the extensive misuse, overuse, and abuse of different antibiotics by healthcare workers or patients themselves who tend to take antibiotics without medical consultation and prescriptions (Ouchar Mahamat *et al.*, 2019, Sokhn *et al.*, 2020).

Imipenem has considered as the furthermost effective antimicrobial agent against hospitalized ESBLs producing isolates. The carbapenem antimicrobial agents like imipenem, ertapenem and meropenem, were frequently known as the first drug of choice in the serious infections' treatment generated by ESBL-producing *Enterobacteriaceae* (Mobasherizadeh *et al.*, 2012, Gholipour *et al.*, 2014).

## CONCLUSION

It is recommended to monitor ESBL *P. mirabilis* producers. Aminoglycosides and carbapenems has been the drug of choice for infections treatment caused by ESBL producing *P. mirabilis*. Though, they must be prescribed cautiously to avoid loss of outer membrane porin that could change the antibiotic access with marked change of the sensitivity pattern.

**Declaration of interest: none**

Author Contributions: Lamiaa A. Salama: Methodology, Visualization, Investigation, Writing- Original draft preparation. Hazem Hamed Saleh: Methodology, Investigation. Shaymaa H. Abdel-Rhman: Visualization, Investigation, Writing- Reviewing and Editing. Rasha Barwa: Conceptualization, Visualization, Writing- Reviewing and Editing. Ramadan Hassan: Supervision, Writing-Reviewing and Editing.

This article had not been issued anywhere or currently under consideration by another journal or a publisher.

## References


Ali, H. H. and Yousif, M. G. (2015): Detection of some virulence factors genes of *Proteus mirablis* that isolated from urinary tract infection. *IJAR*; 3 (1): 156-163.

Armbruster, C. E., Mobley, H. L. T. and Pearson, M. M. (2018): Pathogenesis of *Proteus mirabilis* Infection. *EcoSal Plus*; 8 (1): 10.1128/ecosalplus.ESP-0009-2017.

Chanal, C., Bonnet, R., De Champs, C., Sirot, D., Labia, R. and Sirot, J. (2000): Prevalence of β-lactamases among 1,072 clinical strains of *Proteus mirabilis*: a 2-year survey in a French hospital. *Antimicrobial agents and chemotherapy*; 44 (7): 1930-1935.

CLSI (2017). Performance standards for antimicrobial susceptibility testing, Clinical and Laboratory Standards Institute Wayne, PA.

Danilo de Oliveira, W., Lopes Barboza, M. G., Faustino, G., Yamanaka Inagaki, W. T., Sanches, M. S., Takayama Kobayashi, R. K., Vespero, E. C. and Dejato Rocha, S. P. (2021): Virulence, resistance and clonality of *Proteus mirabilis* isolated from patients with community-acquired urinary tract infection (CA-UTI) in Brazil. *Microb Pathog*; 152: 104642.

Gajdács, M. and Urbán, E. (2019): Comparative Epidemiology and Resistance Trends of Proteae in Urinary Tract Infections of Inpatients and Outpatients: A 10-Year Retrospective Study. *Antibiotics*; 8 (3): 91.

Gharrah, M. M., Mostafa El-Mahdy, A. and Barwa, R. F. (2017): Association between Virulence Factors and Extended Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* Compared to Nonproducing Isolates. *Interdisciplinary Perspectives on Infectious Diseases*; 2017: 7279830.

Gholipour, A., Soleimani, N., Shokri, D., Mobasherizadeh, S., Kardi, M. and Baradaran, A. (2014): Phenotypic and molecular characterization of extended-spectrum β-lactamase produced by *Escherichia coli*, and *Klebsiella pneumoniae* isolates in an educational hospital. *Jundishapur journal of microbiology*; 7 (10): e11758.

Gravey, F., Loggia, G., de La Blanchardière, A. and Cattoir, V. (2017): Bacterial epidemiology and antimicrobial resistance profiles of urinary specimens of the elderly. *Médecine et Maladies Infectieuses*; 47 (4): 271-278.

Huang, Y., Xu, Y., Wang, Z. and Lin, X. (2014): Antimicrobial resistance and genotype analysis of Extended-Spectrum-β-Lactamase-Producing *Proteus mirabilis*. *Open Journal of Clinical Diagnostics*; 2014: 57-56.

Ishikawa, K., Matsumoto, T., Yasuda, M., Hattori, R., Uehara, S., Muratani, T., Yagisawa, M., Sato, J., Niki, Y. and Totsuka, K. (2011): The nationwide study of bacterial pathogens associated with urinary tract infections conducted by the Japanese Society of Chemotherapy. *Journal of Infection Chemotherapy*; 17 (1): 126-138.

Jain, A. and Mondal, R. (2008): TEM & SHV genes in extended spectrum β-lactamase producing Klebsiella species & their antimicrobial resistance pattern. *Indian Journal of Medical Research*; 128 (6): 759-764.

Karimisup, A., Rahbar, M., Fallahsup, F., Navidiniasup, M. and Malekansup, M. A. (2012): Detection of integron elements and gene groups encoding ESBLs and their prevalence in Escherichia coli and Klebsiella isolated from urine samples by PCR method. *African Journal of Microbiology Research*; 6 (8): 1806-1809.

Kaur, J., Chopra, S. and Sheevani, G. M. (2013): Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Journal of clinical and diagnostic research: JCDR*; 7 (2): 229.

Korytny, A., Riesenberg, K., Saidel-Odes, L., Schlaeffer, F. and Borer, A. (2016): Bloodstream infections caused by multi-drug resistant *Proteus mirabilis*: Epidemiology, risk factors and impact of multi-drug resistance. *Infectious Diseases*; 48 (6): 428-431.

Kurihara, Y., Hitomi, S., Oishi, T., Kondo, T., Ebihara, T., Funayama, Y., Kawakami, Y. and Chemotherapy (2013): Characteristics of bacteremia caused by extended-spectrum beta-lactamase-producing *Proteus mirabilis*. *Journal of Infection*; 19 (5): 799-805.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T. and Monnet, D. L. (2012): Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*; 18 (3): 268-281.

Mata, C., Navarro, F., Miró, E., Walsh, T. R., Mirelis, B. and Toleman, M. (2011): Prevalence of SXT/R391-like integrative and conjugative elements carrying bla CMY-2 in *Proteus mirabilis*. *Journal of antimicrobial chemotherapy*; 66 (10): 2266-2270.

Mobasherizadeh, S., Shokri, D., Zargarzadeh, A. H., Jalalpour, S., Ebneshahidi, S. A. and Sajadi, M. (2012): Antimicrobial resistance surveillance among hospitalized and non-hospitalized extend-spectrum beta-lactamase producing *Escherichia coli* from four tertiary-care hospitals in Isfahan, Iran; 2008-2011. *African Journal of Microbiology Research*; 6 (5): 953-959.

Nakama, R., Shingaki, A., Miyazato, H., Higa, R., Nagamoto, C., Hamamoto, K., Ueda, S., Hachiman, T., Touma, Y. and Miyagi, K. (2016): Current status of extended spectrum β-lactamase-producing *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* in Okinawa prefecture, Japan. *Journal of Infection and Chemotherapy*; 22 (5): 281-286.

Nakano, R., Nakano, A., Abe, M., Inoue, M. and Okamoto, R. (2012): Regional outbreak of CTX-M-2 β-lactamase-producing *Proteus mirabilis* in Japan. *Journal of medical microbiology*; 61 (12): 1727-1735.

Ouchar Mahamat, O., Lounnas, M., Hide, M., Dumont, Y., Tidjani, A., Kamougam, K., Abderrahmane, M., Benavides, J., Solassol, J., Bañuls, A.-L., jean-Pierre, H., Carrière, C. and Godreuil, S. (2019): High prevalence and characterization of extended-spectrum ß-lactamase producing Enterobacteriaceae in Chadian hospitals. *BMC Infectious Diseases*; 19 (1): 205.

Pagani, L., Dell'Amico, E., Migliavacca, R., D'Andrea, M. M., Giacobone, E., Amicosante, G., Romero, E. and Rossolini, G. M. (2003): Multiple CTX-M-type extended-spectrum β-lactamases in nosocomial isolates of Enterobacteriaceae from a hospital in northern Italy. *Journal of Clinical Microbiology*; 41 (9): 4264-4269.

Pawar, S., Ashraf, M. I., Mujawar, S., Mishra, R. and Lahiri, C. (2018): In silico Identification of the Indispensable Quorum Sensing Proteins of Multidrug Resistant *Proteus mirabilis*. *Frontiers in cellular and infection microbiology*; 8: 269-269.

Quinteros, M., Radice, M., Gardella, N., Rodriguez, M., Costa, N., Korbenfeld, D., Couto, E. and Gutkind, G. (2003): Extended-spectrum β-lactamases in Enterobacteriaceae in Buenos Aires, Argentina, public hospitals. *Antimicrobial Agents and Chemotherapy*; 47 (9): 2864-2867.

Saurina, G., Quale, J. M., Manikal, V. M., Oydna, E. and Landman, D. (2000): Antimicrobial resistance in Enterobacteriaceae in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns. *Journal of Antimicrobial Chemotherapy*; 45 (6): 895-898.

Shin, J., Ko, K. S. and chemotherapy (2014): Comparative study of genotype and virulence in CTX-M-producing and non-extended-spectrum-β-lactamase-producing *Klebsiella*

*pneumoniae* isolates. *Journal Antimicrobial agents*; 58 (4): 2463-2467.

Sokhn, E. S., Salami, A., El Roz, A., Salloum, L., Bahmad, H. F. and Ghssein, G. (2020): Antimicrobial susceptibilities and laboratory profiles of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates as agents of urinary tract infection in Lebanon: Paving the way for better diagnostics. *Medical Sciences Journal*; 8 (3): 32.

Tambekar, D., Dhanorkar, D., Gulhane, S., Khandelwal, V. and Dudhane, M. (2006): Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. *African Journal of Biotechnology*; 5 (17): 1562-1565.

Wang, J.-T., Chen, P.-C., Chang, S.-C., Shiau, Y.-R., Wang, H.-Y., Lai, J.-F., Huang, I.-W., Tan, M.-C. and Lauderdale, T.-L. Y. (2014): Antimicrobial susceptibilities of *Proteus mirabilis*: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. *BMC infectious diseases*; 14 (1): 486.