

Original Research Article

Frequency of extended-spectrum beta-lactamase (ESBL) – multidrug resistance produced by *Pseudomonas aeruginosa* isolated from clinical specimens in Khorramabad City, Iran

Abstract:

Background:

Pseudomonas aeruginosa is one of the most important pathogenic bacteria causing hospital infections, which has intrinsic resistance to many antibiotics. One of the reasons for the emergence of drug resistance in *p.aeruginosa* isolates is the production of ESBLs (extended-spectrum beta-lactamase) enzymes. Resistance rate is increasing due to the production of these enzymes in *p.aeruginosa*. The goal of this study was to determine the prevalence of ESBLs enzymes in *p.aeruginosa* isolates from different samples of patients in Khorramabad city by CDT (combined disk test) phenotypic method.

Comment [u1]: Capitalize letter P before italicizing it. Applicable to all part of this manuscript.

Methods:

This study was an investigation on 70 *p.aeruginosa* samples isolated from different samples of patients in medical centers of Khorramabad city during one year. The bacteria were identified by routine biochemical tests. Antibiotic sensitivity of the isolates was evaluated by double disk diffusion method. Phenotypic investigation of ESBLs production among the tested isolates was performed by cefotaxime disks and ceftazidime both alone and in combination with clavulanic acid (combined disk test).

Results:

Results of combined disk test showed that, out of 70 isolated pseudomonas, 100% of the samples had multi-drug resistance. Maximum resistance to cefixime (79.1%) and lowest resistance to meropenem (25%) was observed. and 25 (35.7%) were phenotypic beta-lactamase-producing enzymes. That 35% of the strains About 17.1% of the wounds were beta-lactamase producers.

Comment [u2]: reconstruct

Comment [u3]: about

Conclusion:

Considering the increasing prevalence of *p. aeruginosa* strains which produce extended-spectrum beta-lactamase, it is recommended to use an appropriate treatment protocol based on determining antibiogram pattern of strains. This means that all isolated before treatment with antibiotics by antibiotic susceptibility testing be examined and be used the antibiotics accordance with the CLSI (Clinical and Laboratory Standards Institute) standards. This issue is a serious alarm for applying infection-control criteria with the purpose of preventing the following spread of this microbe.

Comment [u4]: let your grammer flow pls.

Keywords: Disk Diffusion Antimicrobial Tests, *Pseudomonas aeruginosa*, Multi drug resistant, extended-spectrum beta-lactamase

1. Introduction:

P. aeruginosa is one of the most important pathogenic bacteria, which causes hospital infections like pneumonia, bacteremia, and urethritis infections. This bacterium is one of the important factors of chronic pulmonary infections and death in children and adults with cystic fibrosis disease (1). Also, it is an opportunist pathogenic bacterium which is a major cause of mortality in patients with impaired immune system (1). As a result of clinical consumption of antibiotics, the prevalence of hospital *p.aeruginosa* strains which are multi-drug resistant (MDR) has been increased worldwide and turned into a serious problem in hospital management (1, 2). Intrinsic resistance to antimicrobial agent in this bacterium exacerbates treatment condition of its infections (1, 2). Resistance mechanisms of *p. aeruginosa* include producing beta-lactamase, secretive pumps, and external membrane changes (3). Resistance to various drugs is usually the result of a combination of different mechanisms in one strain or function of a specific mechanism. A type of resistance which appears due to the production of enzymes called beta-lactamase is the resistance factor to beta-lactamase (3). Beta-lactamase was an appropriate drug for treating *p. aeruginosa*; but, after a while, some of the bacteria showed resistance to these antibiotics by producing beta-lactamase (3). Resistance of *p. aeruginosa* strains to the second and third generation (extended-spectrum) cephalosporins and monobactams is done through extended-spectrum beta-lactamase enzymes (ESBLs) (4, 5). These enzymes are coded by different genes which are placed on the chromosome or plasmid (4, 5). Extended-spectrum beta-lactamases (ESBLs) are of type A beta-lactamase (5, 6) and include a number of evident ESBL specifications which inhibit their activity in the presence of clavulanic acid (beta-lactamase inhibitor). So, this specification is used as a confirmatory test in tracing ESBLs in laboratory (6) so that the bacterial strain which is resistant to one of the third generation cephalosporins (cefotaxime, ceftriaxone, cefixime, and ceftazidime and its beta-lactamase is stopped in the presence of clavulanic acid) is considered as positive ESBLs. Thus, the treatment of infections caused by this bacterium fails despite its easy laboratory detection (6,7). The most conventional ESBLs reported from western and Asian countries are extended-spectrum beta-lactamase derived from SHV and TEM which are placed on large plasmids and sometimes transferred to different strains with one genus or other genera of enterobacteriaceae family along with resistance indices (7). Nowadays, indiscriminate use of antibiotics in Iran has led the bacteria to gain resistance mechanisms and show resistance to drugs (2). Considering the mentioned points and due to the role and importance of strains in uncontrollable hospital infections, this research considered the *p. aeruginosa* isolated from clinical samples in Khorramabad city.

2. Materials and Methods:

This research was a cross-sectional study. 70 samples of *p. aeruginosa* were isolated from different clinical samples such as wound, blood, pulmonary secretions and mucus, urine, tissue, body liquids, and cerebrospinal fluid in different medical centers of Khorramabad city. of the 70 samples were collected 30 cases of wounds, urine (19), pulmonary secretions (8), blood (6), tissue (4) and the fluid (3), respectively. Identification and determination of bacterial identity were done by routine discriminative biochemical tests; including manner of sugar fermentation in TSI (triple sugar iron) agar medium, lysine iron agar decarboxylation in lysine iron agar medium LIA (lysine iron agar), indole production and motility in SIM (SH₂, indole, motility) medium, reaction in VP (Voges Proskauer) and MR (Methyl Red) media, and consumption of Simmon's citrate medium, growth in urea agar, and fermentation of different sugars (glucose, maltose, cellobiose, mannitol, and sucrose) in OF medium (1, 2, 4). Finally, the results were compared using standard tables. Drug sensitivity test was done by disk diffusion method based on CLSI (Clinical and Laboratory Standards Institute) instructions on Mueller Hinton agar medium. Antibigram results for screening ESBLs and MDR samples after 18-24 hrs of incubation at 37°C were read by the above-mentioned standard institute tables (1, 2, 4). The antibiotic disks used in this study (PadanTeb Company, Tehran, Iran) include tetracycline (30µg), meropenem (10µg), cefotaxime (30µg), ceftazidime(30µg), piperacillin(100µg), ceftriaxone(30µg), ciprofloxacin(5µg), cefixime(30µg), chloramphenicol(30µg), erythromycin(15µg), and gentamicin(10µg).

2.1 Screening beta-lactamase producer samples (ESBLs):

Comment [u5]: reference

Comment [u6]: of the causative agent of

Comment [u7]: capitaliz

Comment [u8]: delete

Comment [u9]: 30 isolates of *P. aeruginosa* was identified from wound source, urine,

Comment [u10]: delete

Comment [u11]: ?

This test was done by disk diffusion method (Kirby–Bauer) based on CLSI standards. At first, after was prepared using Mueller Hinton agar medium, standard microbe suspension was prepared with half McFarland concentration (1, 2, 3, 4). After complete dispersion of the microbial suspension on the mentioned medium, ceftazidime, cefotaxime, and piperacillin antibiotic disks along with their three combined disks with clavulanic acid (ceftazidime/clavulanicacid, cefotaxime /clavulanic acid, piperacillin/tazobactam) were placed on the medium with the distance of 25 mm (figure 1). After 24 hrs incubation of the plates at 37°C, the diameter of inhibition zone around the disk was measured. Increased inhibition zone of equal to or more than 5 mm in the plate containing clavulanic acid disk was determined as ESBLs producer strain (1, 2).

Comment [u12]: rephrase

Comment [u13]: at

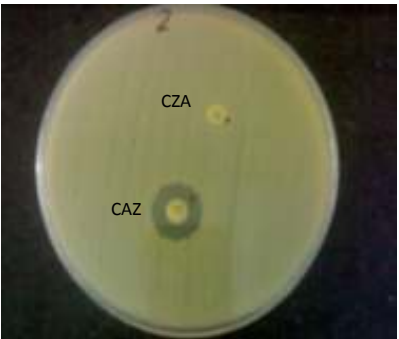


Fig 1.combined disks test with ceftazidime(CAZ) and ceftazidime/clavulanicacid(CZA) disk for Screening beta-lactamase producer samples (ESBLs), in Khorramabad City, Iran

3. Results:

3.1 Microbial sensitivity pattern for MDR strains

Microbial sensitivity pattern for MDR strains was as follows: out of 70 strains, 79.1%, 33.3%, 66.6%, 25%, 58.3%, 66.6%, 45.8%, 34.2%, 58.7%, and 33.3% showed resistance to cefixime, ceftriaxone, gentamicin, meropenem, cefotaxime, ciprofloxacin, ceftazidime, tetracycline, erythromycin, and chloramphenicol, respectively (table 1). According to the obtained results, it was found that 100% of the isolates had multi-drug resistance (table 1). Maximum resistance to Ciprofloxacin was observed in pulmonary secretions. The highest resistance to cefixime in cases of wound.

Comment [u14]: was identified in wound sample

Comment [u15]: delete

Table 1. Resistance Percent of multidrug resistance *P. aeruginosa* isolated from clinical specimens city of Khorramabad, Iran, to various antibiotics

Antibiotics	Percent of resistance
Cefixime(5µg)	79.1%

Ceftriaxone(30µg)	33.3%
Gentamicin(10µg)	66.6%
Meropenem(10µg)	25%
Cefotaxime	58.3%
Ciprofloxacin(5µg),	66.6%
Ceftazidime(30µg)	45.8%
Tetracycline(30µg)	34.2%
Erythromycin(15µg)	58.7%
Chloramphenicol(30µg)	33.3%

3.2 Frequency of ESBLs producer strains:

25 (35.7%) and 45 (64.3%) out of 70 tested *p.aeruginosa* isolates were positive and negative ESBLs phenotypes, respectively. Most beta-lactamase production in clinical specimen was found in the wound. That 35% of the strains About 40% of the wounds were beta-lactamase producers. ESBLs frequency in different clinical samples in this study were as follows. Of 30 Wounds, 19 of urines, 8 pulmonary secretions 6 bloods, 4 tissues and 3 body fluids, the frequency distribution was 17.1%, 8.5%, 4.2%, 2.8%, 1.4%, 1.4% respectively (table 2).

Comment [u16]: space, about

Comment [u17]: w

Table 2: Frequency of ESBLs producer *P. aeruginosa* strains isolated from clinical specimens in Khorramabad, Iran

Comment [u18]: :

Frequency of ESBL(%)	Out of 70	Samples
17.1%	12	Wound(30)
8.5%	6	Urine(19)
4.2%	3	pulmonary secretions (8)
2.8%	2	Blood(6)
1.4%	1	Tissue(4)
1.4%	1	Body fluid(3)
35.7%	25	Total

4. Discussion and conclusion:

MDR *p.aeruginosa* is the most important risk factor in hospital centers. Nowadays, the problem of resistance to beta-lactamase drugs and extended-spectrum cephalosporins is becoming a serious problem and we envisaged that in near future, the world will need new antibiotics to replace the existing once (1, 2, 4). Plasmids which are responsible for ESBLs production carry resistance genes to other antibiotics like aminoglycosides (8). So, there is a limiting selection of appropriate antibiotics for the treatment of ESBLs producer isolates (8). Carbapenems are a selection for infections caused by ESBLs producer isolates; but, the isolates resistant to carbapenem have been also reported like this present research (4). the misuse of antibiotic has indeed influenced the occurrence of resistant strains through direct and indirect mechanisms (2). In this study, the prevalence of MDR *p. aeruginosa* was 100%, which showed a higher percent than other studies. Microbial sensitivity pattern for MDR strains was as follows: out of 70 strains, 79.1%, 33.3%, 66.6%, 25%, 58.3%, 66.6%, 45.8%, 34.2%, 58.7%, and 33.3% showed resistance to cefixime, ceftriaxone, gentamicin, meropenem, cefotaxime, ciprofloxacin, ceftazidime, tetracycline, erythromycin, and chloramphenicol, respectively (table 1). Among the strains, maximum percent of *p. aeruginosa* isolates existed in wound and urine samples; consequently, maximum rate of resistance was observed in these samples. Maximum resistance to Ciprofloxacin was observed in pulmonary secretions. The highest resistance to cefixime in cases of wound. This issue demonstrated that maximum resistance was in the samples isolated from hospitalized patients, which indicated increased antibiotic resistance in hospital environments. Increased acquisition of ESBL genes through horizontal gene transfer, which influences long-term hospitalization can be associated of extended-spectrum beta-lactamase and long-term hospitalization of patients could lead to considerable dispersion of ESBLs producer bacteria (9). The notable point is about the frequency of sample; in the present work, the maximum number of isolates and resistance was found in wounds, which was in agreement with the study at Shahid Beheshti Hospital, Kashan, Iran (1). In this research, maximum rate of resistance was in cefixime (79.1%) and minimum resistance belong to meropenem (25%), which was almost in agreement with the previous studies (1, 5). In a research in Saudi Arabia, the prevalence of ESBLs pseudomonas was 41.6%, which was not in parallel with percent of ESBLs in the present study (5). In studies conducted by Woodford et al in the UK, the prevalence of ESBLs producer strains of *P. aeruginosa* was 3.8%; 4.2% in Malaysian and 7.2% in UK as reported by Jacobson (10,11). While other researchers' results indicate high levels of ESBLs in strains of *P. aeruginosa* e.g. Thailand (28%), India (20.3%) and 39.4% in Tehran burn hospital (12, 13, 14, 15, 16,17). Two hospitals in Tehran in patients with wound infection was 46% that more than the results of this study have been (19). The frequency of ESBLs in different clinical samples as observed in this study include; 30 from wounds, 19 from urines, 8 from pulmonary secretions, 6 from bloods, 4 from tissues and 3 from body fluids. The frequency distribution of ESBLs producer among *P. aeruginosa* strains isolated from clinical specimens in Khorramabad, Iran were 17.1%, 8.5%, 4.2%, 2.8%, 1.4%, 1.4% for wound, urine, pulmonary secretions, blood, tissue and body fluid respectively, while the prevalence rate was 35.7% (table 2). Different reports from various geographic regions approve the role of regional and specific studies on resistance emergence (10). It should be noted that appearance of different types of antibiotic-resistant and ESBL producer strains has been associated with excessive and long-term consumption of broad spectrum cephalosporins, according to most of the reports. The prevalence of these organisms can be reduced through the use of infection control methods and limiting use of oxyimino-cephalosporins (9). Change in antibiotics consumption strategy and high aseptic conditions in areas where patients are hospitalized for long-term, laboratory antibiotic susceptibility testing according to the CLSI standards are also important factor towards controlling microbial drug resistance.

REFERENCES:

1-Tavajjohi Z, Moniri R, Khorshidi A, Frequency of extended-spectrum beta-lactamase (ESBL) multidrug resistance produced by *Pseudomonas aeruginosa* isolated from clinical and environmental specimens in Kashan Shahid Beheshti hospital, *Iranian Journal of Public Health*, 2010; 1390:15(2), 139-1451.

Comment [u19]: This is not multidrug resistance pattern pls. this is percentage resistance of the isolates to selected antibiotics used in Iran. check references the way it been presented.

Comment [u20]: Small letter

Comment [u21]: Elaborate please

Comment [u22]: to

Comment [u23]: delete. Induced by long-term hospitalization of patient (nosocomial infection).

Comment [u24]: Is the patient diabetics? If not why the resistance

Comment [u25]: italicized

Comment [u26]: rephrase or delete

Comment [u27]: reconstruct

Comment [u28]: reference

Comment [u29]: how?

Comment [u30]: Recheck all your references, and correlate it with this journal format.

2-Imani A, rostami Z, Prevalence of antibiotic resistance and ESBLs in *pseudomonas aeruginosa* isolated from clinical specimens phenotypic and genotypic methods, *Iranian Journal of Ardabil University of Medical Sciences*, 2010; 10(3): 189-198

3-Amir Mozaffari N, Forouhesh Tehrani H, and et al, A Survey of Drug Resistance Due to Extended Spectrum Beta Lactamases (ESBLs) in *Escherichia Coli* Strains Isolated from Hospitalized Patients, *Iranian Journal of Medical Sciences*, 2008; 15(59).

4-Hosein zadegan H, hasani M and et al, Identification of broad-spectrum beta-lactamase-producing Gram-negative bacteria isolated from clinical cases, *Journal of Laboratory Medicine*, 2007; 1(2): 20-25

5-Sibhghatulla Shaikh a, Jamale Fatima and et al, Prevalence of multidrug resistant and extended spectrum beta-lactamase producing *Pseudomonas aeruginosa* in a tertiary care hospital; DOI: 10.1016/j.sjbs.2014.06.001

6-Mozzafari N, foruhesh H, Study of drug resistance due to *ESBL Klebsiella pneumoniae* in patients with multi-drug resistant, *Journal of Medical Research*, 2007; 31(3): 241-245

7-Niitsuma K, Saitoh M, Kojimabara M, Kashiwabara N. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated in Fukushima prefecture. *Jpn journal Antibiot*. 2001; 54(2): 79-87.

8-Montie TC, Drake D, Sellin H, Slater O, Edmonds S. Motility, virulence and protection with flagella vaccine against *Pseudomonas aeruginosa* infection. *Antibiot Chemother*. 1987; 39, 233-48.

9-Weldhagen GF, Poirel L, Nordmann P. Ambler class A extended-spectrum beta-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. *Antimicrob Agents Chemother*. 2003; 47(8): 2385-92.

10-Woodford N, Zhang J, Kaufmann ME, Yarde S, Tomas Mdel M, et al. Detection of *Pseudomonas aeruginosa* isolates producing VEB-type extended spectrum β -lactamases in the United Kingdom. *J Antimicrobial Chemotherapy* 2008; 62: 1265-68.

11-Lim KT, Yasin RM, Yeo CC, Puthucheary SD, Balan G, Maning N, et al. Genetic fingerprinting and antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* hospital isolates in Malaysia. *J Microbial Immunol Infect* 2009; 42:197 -209

12-Jacobson KL, Cohen SH, Inciardi JF, King JH, Lippert WE, Iglesias T, et al. The relationship between antecedent antibiotic use and resistance to extended spectrum cephalosporins in Group I beta-lactamase producing organisms. *Clin Infect Dis*, 1995; 21(5): 1107

13-Mirsalehian A, Feizabadi M, AkbariNakhjavani F, Jabalameli F, Goli H, Kalantari N. Detection of VEB-1, OXA-10 and PER-1 genotypes in extended spectrum beta-lactamase-producing *Pseudomonas aeruginosa* strains isolated from burn patients. Burns 2010; 36(1): 70-4.

14- Aggarwal R, Chaudhary U, Bala K. Detection of extended spectrum beta-lactamase in *Pseudomonas aeruginosa*. Indian J Pathol Microbiol 2008; 51(2): 222-24.

15-Ulah F, Malik SA, Ahmed J. Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan. Burns 2009; 35(7): 1020-25.

16-Luilitanond A, Kaewkes W, Kitcharoen K. Extended-spectrum β -lactamases (ESBLs) from Gram negative bacilli isolated from Srinagarind Hospital, Thailand. Clin Microbiol Infect 1997; 3 Suppl 2: 300.

17-Shahcheraghi F, Nikbin VS, Shouraj F. PCR Detection of PER & VEB & SHV and TEM beta lactamase in Multidrug resistant *P. aeruginosa* Isolated from Wound Infections in Two Hospital of Tehran. Iran J Med Microbiol 2008; 1(4): 21-7.

18-Masjedian F, Valehi F, Talebi A, RastegarLari A. Evaluation of wide broad spectrum antibiotic resistance of *E. coli* and *Klebsiella pneumoniae*. Iran J Med Microbiol. 2007; 1 (2) :27-34

19-Knothe H, Shah P, Kremery V. Transferable resistance to cefotaxime, ceftazidime, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection. 1983; 11(6): 315-7.

20-Jacoby GA, Medeiros AA. More extended spectrum β -lactamases. Antimicrob Agents Chemother. 1991; 35(9): 1697-1704