Original Research Article

Frequency of extended-spectrum beta-lactamase (ESBLs) –multidrugresistanceproduced by *Pseudomonas aeruginosa*isolated from clinical specimens inKhorramabad 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 ESBLsenzymes in *P.aeruginosa*isolates from different samples of patients in Khorramabad city by CDT(combined disk test) phenotypic method.

Methods:

This study was an investigation on 70 *P.aeruginosa* samples isolated frompatients in medical centers of Khorramabad city during one year(2013). 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 usingcefotaxime and ceftazidimedisks alone and in combination with clavulanic acid (combined disk test) was performed.

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 minimum resistance to meropenem (25%) were observed. Out of 70 isolates, 25 (35.7%) were phenotypic beta-lactamase-producing enzymes. That highest percentage was related to wounds, equal to 12 (17%).

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 treating with antibiotics were examined by antibiotic susceptibility test. Also antibioticsshould be usedaccording toCLSI (Clinical and Laboratory Standards Institute). This issue is of serious concern for applying infection-control criteria with the purpose of preventingspread of this microbe.

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(1). This bacterium is one of the causative agentsofchronic pulmonary infections and death in children and adults with cystic fibrosis disease (1). Also, it is an opportunistic pathogenic bacterium which is a major cause of mortality in patients with animpaired 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 antimicrobialagents in this bacterium exacerbates treatment conditions of its infections (1, 2). Resistance mechanisms of *P.aeruginosa* include producing beta-lactamase, secretion pumps, and external membrane changes (3). Resistance to various drugs is usually the result of a combination of different mechanisms in one strain or thefunction of a specific mechanism.Kindofresistancedue to theproduction ofbeta-lactamaseenzymesis the resistance factor to beta-lactamase (3). Beta-lactamase has been an appropriate drug for treating *P. aeruginosa*; but, after a while, some of the bacteria show 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 iscused by extended-spectrum beta-lactamase enzymes (ESBLs) (4, 5). These enzymes are coded by either genes of chromosomal or plasmid origin(4,5). ESBLs are type A beta-lactamases (5, 6) and include a number of evident ESBL specifications which inhibit their activity in the presence of clavulanic acid (beta- lactamase inhibitor). This specification is used as a confirmatory test in tracing ESBLs in laboratory (6).Therefore,bacterial strainsresistantto one of the third generation cephalosporins (cefotaxime, ceftriaxone, cefixime, and ceftazidime) and its beta-lactamase is stopped in the presence of clavulanicacidis.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 ESBLs derived from SHV and TEM which are placed on large plasmids and sometimes transferred to different strains with one genus or other genera oftheenterobacteriaceae family along withresistance 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, the aim of this research was surveyof *P.aeruginosa*isolated from clinical samples in Khorramabad city, Lorestan province, Iran.

2. Materials and Methods:

This cross-sectional study was conducted on 70 samples of *P.aeruginosa*that were isolated from different clinical samples such as wounds, blood, pulmonary secretions and mucus, urine, tissues, body liquids, and cerebrospinal fluid in different medical centers of Khorramabad city. Identification and determination of bacterial identity were done by routine discriminative biochemical tests,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 (SH_2, indole, motility) medium, reaction in VP (VogesProskauer) 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. Antibiogram results for screening ESBLs and MDR samples after 18-24 hrs of incubation at 37ºC were read by the above-mentioned standard institute tables (1, 2, 4). The antibiotic disks used in this study (PadtanTebCompany, Tehran, Iran) includetetracycline(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):

This test was done by thedisk diffusion method (Kirby–Bauer) based on CLSI standards. At first standard microbe suspension was prepared with half McFarland concentration,and cultured on Mueller-Hinton agar medium.(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 at the distance of 25 mm(figure 1). After 24 h incubation of the plates at 37ºC, the diameter of theinhibition zone around the disk was measured. AnIncreased 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).

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:

Out of the 70 samples collected, 30 isolates of *P. aeruginosa* was identified from wounds source, urine (19), pulmonary secretions (8), blood (6),tissues (4) and the fluid (3), respectively.

3.1 Microbial sensitivity pattern for MDR strains

TheMicrobial sensitivity pattern for MDR strains was as listed in Table 1.According to the obtained results, 100% of the isolates showed multi-drug resistance (Table 1).Maximum resistance to ciprofloxacin was observed in pulmonary secretions. The highest resistance was observed forcefiximein.

Table 1.Resistance percent of *P. aeruginosa*isolated from clinical specimensto various antibiotics inKhorramabadcity,Lorestan province, Iran

Antibiotics	Percent of resistance
Cefixime(5µg)	**79.1%**
Ceftriaxone(30µg)	**33.3%**
Gentamicin(10µg)	**66.6%**
Meropenem(10µg)	**25%**
Cefotaxime(30µg)	**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 ESBL producer strains:

Out of 70 tested *P.aeruginosa* isolates,25 (35.7%) and 45 (64.3%) were positive and negative ESBLs phenotypes, respectively.

Most beta-lactamase production in clinical specimen was found in wounds. That 35% of the strains about40% of the wounds were beta-lactamase producers.The ESBL frequency in different clinical samples in this study are listed in Table 2.

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

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 envisage that in thenear future, the world will need new antibiotics to replace the existing ones (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 ESBL producer isolates (8). Carbapenems are a selection for infections caused by ESBL producer isolates; but, the isolates resistant to carbapenem have been also reported like inthisstudy (4). theabuse of antibiotics 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 percentage than inother studies.percentage resistance of the isolates to selected antibiotics used, 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, themaximum percentage of *P.aeruginosa* isolates existed in wound and urine samples; consequently, themaximum rate of resistance was observed in these samples. Maximum resistance to ciprofloxacin was observed in pulmonary secretions. Wound infectionswere most resistant to cefixime.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 associatedwithextended-spectrum beta-lactamase and nosocomial infection could lead to considerable dispersion of ESBL 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 ShahidBeheshti Hospital, Kashan, Iran (1).In this research, maximum rate of resistance was obtained forcefixime(79.1%) and meropenem (25%) showed minimum resistance, which was almost in agreement with previous studies (1, 5). A study in Saudi Arabia, showed a prevalence of ESBL pseudomonas of 41.6%, which was in agreementwith percentage of ESBL in the present study (5). In studies conducted byWoodford *et al*in the UK, the prevalence of ESBLproducer strains of*P.aeroginosa*was3.8%.The percentage of 4.2% in Malaysian and 7.2% in UK individuals, respectively, has been reported(10,11).Meanwhile,other studies indicated high levels of ESBLs in strains of *P. aeruginosa*inThailand (28%), India (20.3%) and burn hospital Tehran (39.4%)(12, 13, 14, 15, 16,17).Thefrequency distribution of ESBL producers among *P. aeruginosa*strains isolated from clinical specimens in Khorramabad, Iranwere 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).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(1,5,10,19). The prevalence of these organisms can cause endemic and epidemic nosocomial infections that subject to certain conditions such as underlying disease, use of invasive devices, and previous use of antibiotics. Nosocomial infectionscan bereducedby the use of infection control methods, such as communicable disease control, sterilization, disinfection, proper use of equipment, application of sanitation, hand washing and limiting the use of ofoxyimino-cephalosporins(9).

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