Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN: 2521-3520 (online)
Volume 28, Issue 1, 2017
DOI: http: //doi.org/10.23851/mjs.v28i1.309

Research Article

Detection of *blaCTX-M* gene among *Pseudomonas aeruginosa* isolated from water samples in Baghdad

Saba R. Khdair[1], Ali M. Hassan[1], H. M. Abdul Wahab[2], Nibras N. Mahmood[1]
[1]Department of Biology, College of Science, Mustansiriyah University, IRAQ.
[2]Institute of Technology, IRAQ.
*Correspondant email: Sabaaaltaii@yahoo.com

ArticleInfo

Received
17/9/2016

Accepted
30/11/2016

Abstract

A total of 50 environmental *Pseudomonas aeruginosa* isolates were collected from sewage and tap water in Baghdad, Iraq. The MICs of Cefotaxime and Ceftazidime were determined by using agar dilution method, The MIC ranged from 2 to 256 µg/ml.The results of antibiotic sensitivity test showed that among sewage P. aeruginosa isolates, resistance was observed most often to Ticarcillin (92%), Penicillin G (84%), Ceftazidime (12%), (8%) for each of Cefotaxime and Ticarcillin. On the other hand, all tap water isolates were sensitive to Ofloxacin and Levofloxacin, Except (5%) of isolates were resistant to Cefotaxime (25%) to Ceftazidime and (95%) to Ticarcillin. All isolates were tested for Extended-Spectrum β-Lactamase (ESBL) production. Ten isolates (20%) were found to be ESBL producers. All environmental P. aeruginosa isolates were screened for the presence of the blaCTX-M genes by application PCR, Only (30%) of them were positive for this test.

Keywords: *Pseudomonas aeruginosa*, Sewage, *blaCTX-M* gene.

الخلاصة

جمعت 50 عزلة من بكتريا*Pseudomonas aeruginosa* من مياه الصرف الصحي ومياه الحنفية في بغداد-العراق. وتم تحديد التركيز المثبط الادنى لكل من المضادات سيفوتاكسيم والسيفنازيديم باستخدام طريقة التخفيف بالاكار. تراوحت قيمة التركيز المثبط الادنى من 2 الى 256 ميكروغرام/مل ، وأظهرت معظم عزلات بكتريا Pseudomonas aeruginos المعزولة من مياه الصرف الصحي مقاومة تجاه المضاد تيكارسيلين (92٪) ، البنسلين (84) G٪ السيفتازيديم (12٪) ، (8٪) لكل من سيفوتاكسيم وتيكارسيلين. من ناحية أخرى كانت جميع عزلات ماء الحنفية حساسة للأوفلوكساسين والليفوفلوكساسين،عدا (5٪) من العزلات كانت مقاومة للسيفوتاكسيم و (25٪) للسيفتازيديم و (95٪) من تيكارسيلين.
تم اختبار جميع العزلات المدروسة لإنتاجESBL (Extended-Spectrum β-Lactamase). تم تحديد 10 عزلات (20٪) منها منتجة للـ ESBL. واخضعت جميع عزلات *Pseudomonas aeruginosa* للتحري عن وجود جينات*blaCTX-M* بواسطة تقنية PCR، واظهرت النتائج ان 30٪ من العزلات كانت حاملة للجين . *blaCTX-M*

Introduction

Pseudomonas aeruginosa is a pathogenic bacterium that has been thoroughly investigated since the 19th century and is generally regarded as a freshwater or terrestrial organism, this bacterium has a remarkable ability to adapt and thrive in a variety of environments: water [1]. Soil occupational places, such as M. *et al.* Working fluids, hospital and municipal wastewater, tap water and water distribution systems [2] [3].

Multidrug-resistant (MDR) strains of P. aeruginosa are isolated from patients suffering from nosocomial infections.Thus infections are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs [4]. Resistance to β-lactam antibiotics dates back to the first years of discovery of resistance to the first antibiotic, penicillin. The first β-lactamase was observed in Escherichia coli bacteria which hydrolyzed penicillin [5].

In recent years, a new family of plasmid-mediated ESBLs, called CTX-M (Cefotaxime-hydrolyzing β-lactamase), has been arisen that preferentially hydrolyzed Cefotaxime. CTX-M was reported in 1989 for the first time in Germany, and is often found in E. coli and

Klebsiella pneumoniae as well as in other Enterobacteriaceae [6].In recent years; CTX-M-type β-lactamases have been recognized as a growing family possessing a high level of hydrolyzing activities, especially against Cefotaxime (CTX) and Ceftriaxone. Nearly 40 variants of the CTX-M-type enzymes have been identified and registered to date [7][8]. The aims of this work were to detection of bla CTX-M among Environmental P.aeruginosa isolates in Baghdad.

Materials and Methods

Bacterial Isolates

A total of 50 isolates of *P.aeruginosa* bacteria were collected from sewage (Al-Rustamiyh) and tap water (Palestine Street) in Baghdad. The isolates were identified by their colony characteristic, Gram-stain and confirmed by using Vitec 2 system [9].

Antibiotic Susceptibility Testing

The antibiotic susceptibility test was done by using Kirby-Bauer disc diffusion technique on Mueller Hinton agar (Oxoid, England) following Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. Isolates were tested against the following antimicrobial agents: Cefotaxime, Ticarcillin, Cefepime, Penicillin G, Aztreoname, Meropenem, Ceftazidime, Oflox-acin and Levofloxacin. The results compared with standard strain *P.aeruginosa* ATCC 154427 from biology laboratory, Mustansiryah University.

Minimal Inhibitory Concentrations

The MICs of Cefotaxime and Ceftazidime were determined by using Mueller-Hinton agar with antibiotic concentrations ranged (2-512) µg/ml according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI) document.

ESBL production by Combined disk test CDT (Phenotypic confirmatory test): A disk of Ceftazidime (30µg) alone and a disk of Ceftazidime + Clavulanic acid (30µg/10µg) were placed indepen¬dently, 30mm apart, on a lawn culture of 0.5 Mc-Farland opacity of the test isolate on Mueller Hinton Agar (MHA) plate and incubated for 18-24 hours at 35°C. An increase of ≥5mm zone of inhibition diameter around the Ceftazi-dime/Clavulanic acid in comparison to Ceftazidime confirmed ESBL production [11, 12].

DNA Preparation and PCR

A PCR reaction with specific primers was performed to identify bla CTX-M gene of each environmental isolate (Table 1) according to Shacheraghi *et al.* [13]. DNA template was prepared as described by Olsvik *et al.* [14]. (25µl) of PCR amplification mixture contained deionized sterile water (12.5µl) Green Go Taq Master Mix pH [8] (Promega, USA)

Table 1: Sequence of forward and reverse primers used for detecting *bla* $_{CTX-M}$ among *P.aeruginosa* isolates.

Primer type	Primer sequence	Product size bp
Forward primer *bla* $_{CTX-M}$	CGCTTTGCGATGTGCAG	550
Reverse primer *bla* $_{CTX-M}$	ACCGCGATATCGTTGGT	

*The protocol for the PCR condition was: 94°C for 45 s, 53°C for 45 s, and 72°C for 60 s, with a cycle number of 32, Gradient PCR (TechNet – 500, USA).

Dendrogram Construction and Genetic Relatedness

Dendrogram for cluster analysis of all the isolates were subjected to evaluation. The dendrogram was constructed on the basis of the banding pattern produced. A binary table or a haplotype matrix for each isolate was constructed by linearly composing presence (1) and absence (0), data derived from analysis of the gel/ antibiogram was subjected for statistical analysis by squared. Euclidean distance is using the software study.

Statistical Analysis

This enabled the plotting of dendrogram showing the level of genetic similarity among the isolates.

Results and Discussion

A total of 50 environmental *P.aeruginosa* isolates were collected from sewage and tap water in Baghdad, Iraq. Table 2 shows the large scale distribution of resistant of bacteria to antibiotics in water is broad applications of antibiotics by humans Persistence of anti-microbial resistant organisms is a growing public health problem in aquatic ecosystem [15]. The

public health significance of large number of the Pseudomonas bacteria found in water is not very clear usually Pseudomonas spp. isolated from water are resistant to antimicrobials [16].

Table 2: Number of environmental *P. aeruginosa* isolates.

Source	Number of isolates	%
Sewage water	20	40
Tab water	30	60
Total	50	

The MICs of Cefotaxime and Ceftazidime were determined using the agar dilution method. MICs of Cefotaxime and Ceftazidime were determined using agar dilution method, Which ranged between 2-256µg/ml. Results of Antibiotic sensitivity showed that among sewage P.aeruginosa isolates, resistance was observed most often to Ticarcillin (92%), Penicillin G (84%), Ceftazidime (12%), (8%) for each of Cefotaxime and Ticarcillin, on the other hand all tap water isolates were sensitive to Ofloxacin and levofloxacin, (5%) of isolates were resistant to Cefotaxime and (25%) to Ceftazidime and (95%) of Ticarcillin (see Table 3). All isolates were tested for ESBL production. Just (10) isolates (20%) were found to be ESBL producers.

Table 3: Susceptibility of Environmental *P.aeruginosa* isolates to antibiotics.

Antimicrobial Agents	Resistance % of Sewage	Resistance % of Tap water
Cefotaxime	8	5
Ticarcillin	92	95
Cefepime	8	5
Penicillin G	84	90
Aztreoname	4	35
Meropenem	8	15
Ceftazidime	12	25
Ofloxacin	4	0
Levofloxacin	0	0

Bali *et al.* [17], mentioned that ESBLs isolates have able to hydrolyze 3rd and 4th generation cephalosporin's and monobactam and the percentage of ESBLs-producing isolates of G-ve were 5.2 %.bacterial species that produce ESBL is an important threat to clinical therapeutics. These organisms elaborate plasmid-encoded β-lactamases, a variety of which have been described among members of the family Enterobacteriaceae. First described in 1983, ESBL producers have contributed to the dramatic increase in recent years to resistance among gram-negative bacteria to β-lactam agents. Plasmid-borne genes code for enzymes that hydrolyze Penicillins, Cephalosporins, and Aztreonam are inhibited by Clavulanic acid [18]. All Environmental *P.aeruginosa* isolates were tested for the presence of the *blaCTX-M* genes by PCR, 30% of *P.aeruginosa* isolates were positive most of them were isolated from sewage (Fig 1). *CTX-M* β lactamases are a class of β Lactamase which have been more recently recognized to preferentially hydrolyze Cefotaxime and were initially reported in the second half of the 1980s. Theses enzymes (Cefotaximases) are a relatively novel family of plasmid mediated extended spectrum Cephalosporins and have be classified under Ambler class [19].

Un like that of TEM or SHV type ESBLs, the population stricter of CTX-M producing isolates is complex and associated with the spread of specific plasmids and/or epidemics. CTX-M type ESBLs have become widely dispersed in many parts of the word and these resistance to Cefotaxime than to Ceftazidime.

Study of Al-Kaabi [20] in Baghdad mentioned that the percentage of bla CTX-M gene was 83.3 %, while *blaCTX-M* gene appeared in all isolates (100%) in *P.aeruginosa* isolates. In the same manner, Al-Margani *et al.* (2013) reported that all of ESBLs and MBLs producer *P.aeruginosa* isolates carried bla CTX-M gene [21]. Al-Margani (2014) showed that the presence of qnr gene in environmental *P.aeruginosa* isolates in Baghdad [22].

Dendrogram for cluster analysis of all the isolates were constructed using dice coefficient values there were two major clusters when dendrogram was generated on the basis of their bla CTX-M genes (A, B) among B group the isolates clusters in to two subgroups (B1 and B2) dendrogram shown 10 isolates unique pattern gene.

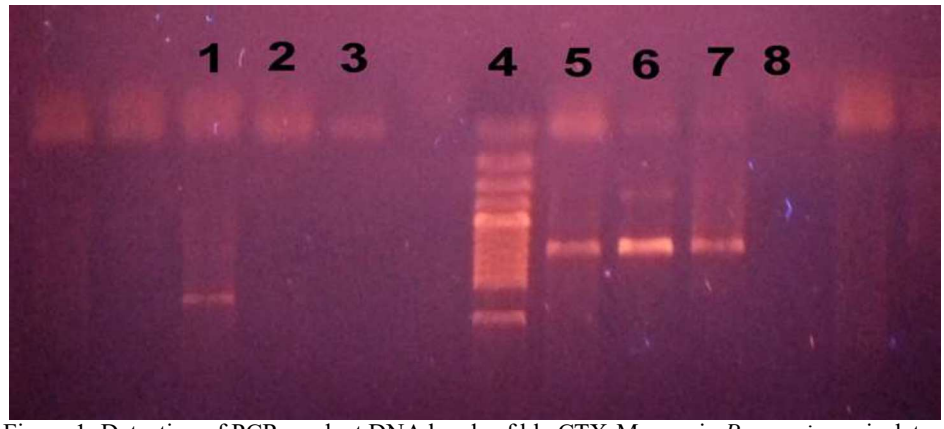
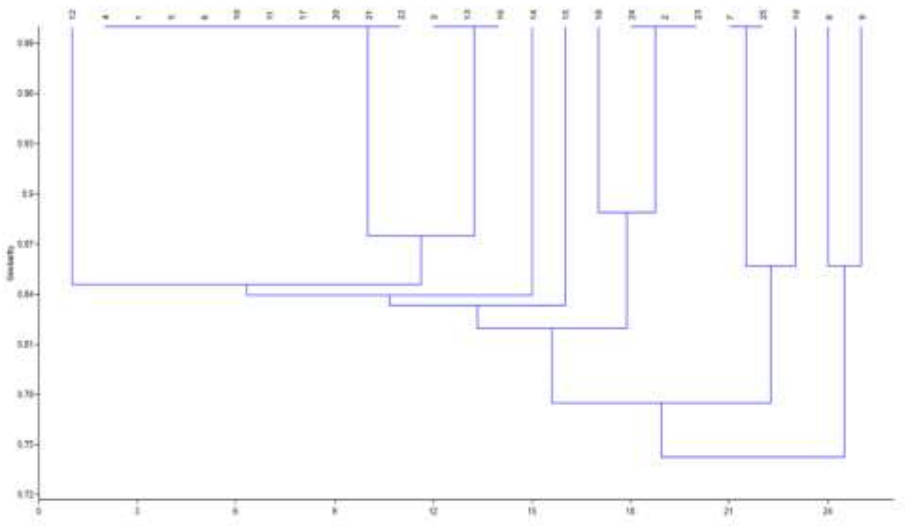


Figure 1: Detection of PCR product DNA bands of bla CTX-M gene in *P.aeruginosa* isolates, (4)=DNA marker. (1, 2, 3, 5, 6, 7, and 8) *P.aeruginosa* isolates.



No. of Isolates

Figure 2: Dendrogram obtained from bla CTX-M gene data for *Pseudomonas aeruginosa*.

These results indicated that the appropriate of dendrogram as a tool for discrimination isolates and to give idea about different same species isolates from the same sources of isolation.This results agreed with Jacome *et al.* (2011) showed molecular typing for the identification of clonal relationship between isolates of P.aeruginosa. The latter has been quite frequented in the recent years for simplicity and efficiency [23]. A close genetic relationship among isolates showed the distribution of organisms in the environment studied. Although some isolates belonged to different clustering groups, they showed more than 80% similarity indicating these isolates originated from a limited number of primary clones. These isolated might tolerate genetic divergence arising from point mutation, insertion or deletion of chromosomal DNA.

References

[1] Lee, C.; Wetzel, TK.; Buckley, I.; Wozniak, D and Lee J. Rapid and Sensitive Detection of *Pseudomonas aeruginosa* in Chlorinated Water and Aerosols targeting gyrB gene using Real-time PCRJ Appl Microbial. October; 111 (4): 893–903. 2011.

[2] Masui A, Zivkovic LI, Fujiwara N. Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of m *et al.* Working fluid. J Biosci Bioeng.; 102: 82–89. 2006.

[3] M, Michalsky T, Wiedeck H, Radosavljevic V, Ruhnke M. Tap water

colonization with *Pseudomonas aeruginosa* in a surgical intensive care unit (ICU) and relation to Pseudomonas infections of ICU patients. Infect Control Hosp Epidemiol.; 22: 49–52. 2001.

[4] Gad, G.F.; El-Domany, R.A.; Zaki, S. and Ashour, H.M. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms, 2007.

[5] Rupp ME, Fey PD. Extended spectrum beta-lactamase ESBL producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. Drugs; 63: 353–365. 2003.

[6] Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev;14: 933–951. 2001

[7] Bonnet, R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. (PMC free article) (PubMed) 48: 1-14. 2004.

[8] Boyd, D. A., S. Tyler, S. Christianson, A. McGeer, M. P. Muller, B. M. Willey, E. Bryce, M. Gardam, P. Nordmann, and M. R. Mulvey. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum β-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob. Agents Chemother. 48: 3758-3764. 2004.

[9] Forbes, B.A.; Sahm, D.F. and Weissfeld, A.S. Baily and Scott' s: Diagnostic Microbiology.12thedition. Mosby, Inc. Baltimore, USA. P: 266-277. 2007.

[10] Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing, informational supplement, CLSI document M100-S19. Wayne, PA: CLSI,.29 (3). 2011.

[11] Bhattacharya, S. Extended spectrum β-lactamases from petridish to the patient. Indian J Med Microbial., 24 (1): 20-24. 2006.

[12] Coudron, P.E., Moland, E. S. and Sanders, C. C. Occurrence and detection of extended-spectrum -lactamases in members of the family Enterobacteriaceae at a veterans medical center: seek and you may find. J. Clin. Microbiol., 35: 2593–2597. 1997.

[13] Shacheraghi, F.; Shakibaie, M.R. and Noveiri, H.Molecular identification of ESBL genes blaGES-1, blaVEB-1, *blaCTX-M* blaOXA-1, blaOXA-4, blaOXA-10 and blaPER-1 in *Pseudomonas aeruginosa* isolated from burn patients by PCR-RFLP and sequencing techniques, International Journal Biological & Life Sciences. 6: 138-142. 2010.

[14] Olsvik, O. and Strockbin, N.A. PCR Detection of Heat-STable, Heat-Label and Shiga-Like toxin genes in Escherichia coli. In. Persing, D.H.; Smith, T.F.; Tenover, F.C. and White, T.J. Diagnostic Molecular Microbilogy. 9th ed. American Society for Microbiology. Washington, DC. 1993.

[15] Reinthaler FF, Posch J, Feierl G, Wüst G, Haas D, Ruckenbauer G, Maschar F, Marth E. Antibiotic resistance of *E.coli* in sewage and sludge. Wat. Res; 37: 1685 - 1690. 2003.

[16] Hernandez-Duquino H, Rosenberg FA. Antibiotic resistant Pseudomonas in bottled drinking water. Can. J. Microbiol;33: 286–289. 1987.

[17] Bali, E. B.; Açık, L. and Sultan, N. Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum β-lactamase produced by Escherichia coli, Acinobacter baumannii and Klebsiella isolates in a Turkish hospital. Afr. J. Microbiol. Res.4: 650-654. 2010.

[18] Altayar, M. A., Thokar, M. and Mohammad, M A. Extended Spectrum B-Lactamase-Producing Escherichia Coli In Clinical Isolates In Benghazi, Libya: Phenotypic Detection And Antimicrobial Susceptibility Pattern Medical Journal of

Islamic World Academy of Sciences, 20: 2, pp. 49-56. 2012

[19] Kingsley J, Verghese S.Sequence analysis of bla CTX28, an ESBL responsible for third generation cephalospo rin resistance in Enterobacteriaceae, for thefirst time in India. Indian J. Pathol. Microbiol.51: 218221. 2008.

[20] Al-Kaabi, M.H.A. Detection of bla TEM, bla SHV, bla CTX-M-1 and bla CTX-M-III genes by using Polymerase Chain Reaction technique from some Gram negative bacteria. M.Sc. Thesis. College of Science. Al-Mustansiryah University. 2011.

[21] Al-Marjani, M.F; Al-Ammar, M.H.M. and Kadhem, E.Q. Occurrence of ESBL and MBL genes in *Pseudomonas aeruginosa* and Acinitibacter bumani isolated from Baghdad, Iraq. International Journal of Current Research, 5 (9): 2482-2486. 2013.

[22] Al-Marjani, M.F. Presence of qnr gene in environmental and clinical *Pseudomonas aeruginosa* isolates in Baghdad. International Journal of Current Microbiology and Applied Science, 3 (7): 853-857. 2014.

[23] Jacome, P.R.; Alives, L.R.; Cabral, A.B. and Maciel, M. Phenotypic and molecular characterization of antimicrobial isolates from Brazil Revista da Sociedade Brasi.trop. 45 (6): 707-712. 2011.