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Detection of Virulance Factors and Antibiotics Susceptibilty of *Pseudomonas aeruginosa* Isolated from Burns and Wounds infections in Ba'quba City.

[*]Ibtihal Hameed Mohsin*, Lina A.S. Al-Saadi **,
Eman sabbah abd al Rahman***
** Department of Biology / College of Science/ Diyala University*
Corresponding Author: Ibtihal Hameed Mohsin

Abstract: *This study was conducted for the period from 1/11/2015 to 15/6/2016 in Baquba city in Iraq. 74 samples were collected from burns with ten isolate (32.2%), wounds infections with six isolate (13..9%) from Baquba General Hospital. The results showed that 15 isolate (93.75%) were able production Lecithenase . Furthermore, 13(81.25%) isolate were able production Haemolysin,and 14 (87.5%) were able production proteinase .The sensitivily of these isolates were tested against (12) antibiotics. The results showed that the highest resistances were for Amoxicillin ,Ampicillin , Cefotaxime ,Trimethoprime with 100% , while the lowest resistance was for Ciprofloxacin 25% , while it showed high sensitivity toward combination Emipenem with 100% .As well as, 11 isolate(68.75%) were able to production β-lactamase., Furthermore, six isolate (37.5%) were able to production metalloβ-Lactamases. Finally, four isolate (25%) were able to production Extended Spectrumβ-Lactamases enzyme*

Conclusion*: p. aeurginosa isolates highest resistances were Amoxicillin, Ampicillin, Cefotaxime ,Trimethoprime with 100%, While the lowest resistance were for Ciprofloxacin 25%. while it showed high sensitivity toward combination Emipenem with 100% . additionally some of isolates production, β-lactamase, metallo β-Lactamases and Extended Spectrumβ-Lactamases enzyme.*

Keywards: *Pseudomonas aeruginosa , β-lactamases, metallo β-Lactamases , Extended Spectrumβ-Lactamases enzyme, Antibiotics .*

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I. Introduction

Pseudomonas aeruginosa is a Gram-negative rod bacterium, which has a remarkable ability to adapt and thrive in a variety of environments: water [(1)] **.** burn , Wounds, endocarditis and otitis externa [(2)] . *P. aeruginosa* has been recognized as a frequent inhabitant of chronic non-healing wounds [(3)]**,** and is one of the foremost opportunistic bacteria isolated from wounds which cause high morbidity and mortality despite antimicrobial therapy [(4)] **.** *Pseudomonas aeruginosa* and other *Pseudomonades* are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed **.** P. aeruginosa produces a number of virulence factors which after colonization can cause extensive tissue damage, bloodstream invasion, and dissemination[(5)] .

Antibiotic resistance in *P. aeruginosa* may be mediated via several distinct mechanisms including modification of site-targeted drugs or outer membranes, β-lactamase production, and efflux pumps. The increase in antibiotic resistance is mostly due to extensive abuse of antibiotics such as ciprofloxacin, β-lactamase and aminoglycosides in the burn centers as well as non-availability and high costs of other effective drugs [(6)] .
Pseudomonas aeruginosa producing metallo-β-lactamase (MBL) was first reported fromJapan in 1991 and since then has been describedfrom various parts of the world including Asia,Europe, Australia, South America , andNorth America [(7)] . Metallo-β-lactamases belong to Ambler class B and have the ability to hydrolyze a wide variety of β- lactam agents, such as penicillins, cephalosporins [(8)] **.**

Phenotyping methods are based on the ability of metal chelators, such as EDTA and thiol-based compounds,inhibit the activity of MBL [(9)] . Double Disk Synergy Test (DDST) and Combined DiscTest (CDT) are most commonly used to detect the MBL producing P. aeruginosa then Hodge test. Modified Hodge test detects only carbapenemase activity, which prevents the use of EDTA and therefore, does not confirm the metal dependence of the carbapenemase [(10)] . Modified Hodge test, DDST and the CDT are easy, reliable, simple to perform and cheaper[(11)].

The extensive use of third and fourth generation cephalosporins as an important component of empirical therapy in intensive care units and high risk wards , resistance to these drugs has became amajor

problem all over the world [12] . Resistance has developed in bacteria by possessing extended spectrum beta – lactamase (ESBLs) capable of hydrolyzing these newer cephalosporins [13,14]. Beta – lactamase mediated resistancemay be overcome by combining beta – lactam antibiotics with beta – lactamase inhibitors which bind irreversibly to the beta – lactamases and render them inactive thus sparing the beta – lactam antibiotic [15] . In 2005 Using of beta-lactamase inhibitors in combination with beta-lactam antibiotics represents an effective measure to combat a specific resistance mechanism of betalactamase producing organisms [15] . In 2001 Three beta-lactamase inhibitors sush as Clavulanic acid, Sulbactam and Tazobactam are in clinical use, and in combination with beta-lactam antibiotics, represent a successful strategy to combat a specific resistance mechanism [16,17,18].

This study was **aimed** to isolate bacterial isolates from burnand wound infections, and test their antibiotic susceptibility pattern against available antibiotics and ability of production β-Lactamases, metallo β-Lactamases , Extended Spectrumβ-Lactamases enzyme in Baquba Hospitals

II. Materials And Methods

Samples collection:

74 clinical samples were collected from patients and carriers in Baquba General Hospital over period from 1/11/2015 to 15/5/2016. The samples.

Sample type	Sample No.	Isolate No. ***P. aeruginosa***	Percentage %
Burns sample	31	10	32.2%
Wounds sample	43	6	13.9%
Average	74	16	21.6%

Isolation and Identification of *P. aeruginosa*:

Samples were cultivated on MacConky's agar, blood agar and Nutrient agar to determine the characteristic features and morphology of the colonies. Gram's stain was done for each sample followed by biochemical tests according to the method described by[19] **.** The isolates were kept on nutrient agar slants in screw- caped test tubes for not more longer than 20 days, the isolates were grown in nutrient broth containing 15% glycerol and stored in deep freeze for longer time maintenance [20] **.** The isolates were identified according to the Bergey's Manual. [21,22] As the following: gram stain and biochemical tests Standard (Oxidase test , Catalase test , Triple sugar iron agar test (TSI),Indol, Methyl red, Citrate utilization and Diagnostic kits**:**API 20E system used for confirmation of suspected some isolates of *P. aeruginosa)* were used for detecting *P. aeruginosa* strains.

Detect of Virulance Factors of *Pseudomonas aeruginosa* :

The ability *P. aeruginosa* to produce some of virulence factors (enzymes and toxins) were recognized and tests were applied on 16 isolates that identified such as:

1. Gelatin liquefaction test [23]. 2. Hemolysin production [23]. 3. Capsule production [23]. 4. Binding to Congo red [23] . 5. Protease production [23] . 6. DNase production [23] . 7. Urease activity [23] . 8. Lecithinase production [23] . 9. Lipase production (Tween 80 hydrolysis test) [24] . 10.β-lactamase production [23] **.**

Antimicrobial susceptibility test:

The sensitivity and resistance of *P. aeruginosa* to antimicrobials was tested by the disc diffusion method on Muellar-Hinton agar using antibiotic discs according to Clinical and Laboratory Standards Institute (CLSI) guidelines. [25] Twelve antibiotics were tested: Ampicillin (25Mg), Penicillin (25Mg), Cefotaxime (30Mg), Erythromycin (15Mg), Ceftazidime (30Mg), Amoxicillin/ Clavulinic acid (30Mg), Gentamicin (10Mg), Ciprofloxacin (5Mg), Piperacillin (30Mg), Amikacin (30Mg), Trimethoprim (10Mg) and Imipenem (10Mg). Interpretation of inhibition zones was carried out based on the manufactures and CLSI guidelines .[25] Then the plates are incubated overnight at 37^{oC}, and the zone of inhibition of bacterial growth is used as a measure of susceptibility, where large zones of inhibition indicate that the organism is susceptible, while small or no zone of inhibition indicate resistance. An interpretation of intermediate is given for zones which fall between the accepted cutoffs for the other interpretations**.** [25]

Detection of β-lactamase, Metalloβ-lactamase(MBL) and extended spectrum β-lactamase (ESBL) Production.

The iodometric method for detection of β-lactamase was described by (WHO) 1978 [26] . Detection of Metalloβ-lactamase by using Imipenem EDTA combined disk test [27] , using two Imipenem disk (10µg) with 3cm between them , and then the (10µg) EDTA solution to one of the drives of Imipenem then incubated at a

temperature 37°C for 18-24 hour, after observing areas of inhibition zone , increase of inhibition zone above 7mm on the disk Imipenem with EDTA compaired with the Imipenem disk alone , the result is positive . Disk Approximation method was performed for detection of ESβLs for all isolates which were positive to β-lactamase production [(28)] .

III. Results And Discussion:

Isolation and Identification:

Isolates that grow on *pseudomonas* ceterimide agar, that suspected to be *P. aeruginosa* were further identified according to their morphological, characteristics and biochemical tests. To do this, each colony that grew on pseudomonas agar was reinoculated on nutrient agar. Certain morphological colonial appearance e.g. mucoidal growth, smooth shaped with flat edges and elevated center, whitish or creamy color, fruity odor and pyocyanin production were undertaken as a pseudomonas indication [(29)] . Microscopical examination of each isolated has shown that they were all having single cells, non spore forming, Gram-negative an rod-shaped bacteria [(30).]

Biochemical tests were done to identify *P. aeruginosa*. These results are all of the isolates gave a positive result for catalase, oxidase, gelatinase, urease and pyocyanin pigment production. Those isolates have utilized glucose and citrate as carbone sources. β- hemolysis produced on blood agar, the isolates hemolyze human red blood cells (RBC_S) which indicates that they are of phospholipase C (which produced by *P. aeruginosa).* These biochemical results are in accord with [31, 32, 33] . who have delt with the study isolation and diagnosis of *P. aeruginosa* from clinical infections of bacterial contaminated.

Except for methyl red test which showed variable results, Tryptophane (Indole), VP tests and H_2S production results were all gave negative results, non-lactose fermentation on macConky's agar. On TSI agar test, 100% gave an alkaline (slant) and red (no change) bottom, due to unability to ferment lactose and sucrose. In addition, ability of this bacterium to grow at 42˚C and 4˚C on *pseudomonas* agar containing ceterimide. These biochemical results were further confirmed by diagnostic key mentioned by Atlas [(34)] , and also by applying API 20E system. However, the present work results were agreed with those observed by (Al- Hilli ; Al- Shaibani, and Al- Gherawi) . [35,36,37]

Antibiotic sensitivity tests:

In the present study, Standard disc diffusion test has used for detection of susceptibility of pathogenic *Pseudomonas* to antibiotics. Ten different antibiotic discs belong to various groups were applied. These included β-lactams, aminoglycosides, cephalosporins, penicillins. Decision whether the isolate is resistant or sensitive to antibiotic was based on the observation and computing the diameter of inhibition zone and then compared them with that of standard value which stated by **NCCLS.** [(38)] However, the results of this work indicated that the isolates of *P. aeruginosa* have demonstrated a higher resistance to Penicillin، Amoxicillin، Erythromycin، Amoxicillin/Clavulinic acid، Trimethoprim that constituted a percentages of 100% this results are agreed with Abdullah et.al. . [(39)] The development of antibiotic resistance is considered a major therapeutic problem that can be explained by some hypothesis such as , the influence of excessive and /or inappropriate antibiotic used [(40)]. *P. aeruginosa* and other *P*seudomonads are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed [(30)] . The results of this work also showed that Cefotaxime, Ceftazidime was effective on *P. aeruginosa* isolates with the percentage of resistance 87.5% and 75% .This result is nearly, in accordance with Qasim[(41)] . percentage of resistance (92%),but this result not agreed with Al-Gherawi (66.7%) . [(37)]

Detection of β-lactamase , Metalloβ-lactamase and ESβLs Production.

Detection of β -Lactamase Enzymes Production The production of β-lactamase enzymes by isolates were determined by rapid iodometric method. the isolates 11 (68.7%) were β-lactamase producer Table.(2). This result was in accordance with the study of Al-Mashhadani [(42)], in which all *P. aeruginosa* isolates recovered from different samples were (100%) β-lactamase producers [(42)] . It was found that the major mechanism of resistance in gram negative bacteria causing clinically significant infection is the expression of ß-Lactamases, of which there are several classes including plasmid encoded and chromosomally encoded enzymes [(43)] .

In Present study, MBL productions was six isolate (37.5%) in isolates of *Pseudomonas* which lower than values reported in other studies in Iran [(44)] , Malaysia [(45)] and Pakistan [(46)] . The multidrug resistant phenotype in *P. aeruginosa* could be mediated by several mechanisms including multidrug efflux systems, enzyme production, outer membrane protein (porin) loss and target mutations. Inappropriate empirical therapy has been associated with increased mortality in *P. aeruginosa* infections; Our study reported 25% ESBL production among *P. aeruginosa* isolates. The studies conducted by others depicted low rates, 3.7% by Woodford et al. [(47)], 4.2% by Lim et al. respectively[(45)] , of ESBL production in *P. aeruginosa* . The results of various studies showed high rate 20.3% by Aggarwal et al. [(48)], 39.41% by Mirsalehian et al. [(44)] and 35.85% by Ullah et al·[(46)] of ESBLs in the samples of *P. aeruginosa* isolates examined.

Table (2): The percentages of *P aeruginosa* isolates producing β-lactamase .

β-lactamase production	Number of isolates	Ratio %
β-lactamase	11	68.75%
Metalloβ-lactamase	6	37.5%
ESBLs	4	25%

References

[1]. Karadzic I, Masui A, Zivkovic LI, Fujiwara N.(2006) Purification and characterization of an alkaline lipase from Pseudomonas aeruginosa isolated from putrid mineral cutting oil as component of metalworking fluid. J Biosci Bioeng.; 102:82–89. [PubMed: 17027868].

[2]. Alammary , M. J.(2013) . Detection of some carbapenem-resistant genes of Pseudomonas aeruginosa isolates from Al-Hilla teaching hospital . reserch College of Science. Babylon .Univ. (M. Sc. Thesis in Microbiology)

[3]. James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J,(2008). Biofilms in chronic wounds. Wound Repair Regen.;16:37-44.

[4]. Lau GW, Hassett DJ, Britigan BE.(2005) Modulation of lung epithelial functions by Pseudomonas aeruginosa. Trends Microbiol;13:389-397.

[5]. (Storey G.D., Ujack E.E., Rabin --H.R. "Population transcript accumulation of Pseudomonas aeruginosa exotoxin A and elastase in sputa from patients with cystic fibrosis". Infect. Immun. 60(11):4687-4694. 1992).

[6]. (Tavajjohi, Z., Moniri, R. and Khorshidi, A. (2011). "Detection and characterization of resistance and extended spectrum β-lactamase producing (ESBL) Pseudomonas aeruginosa isolates in a teaching hospital". African J. Microbiol Res. 5(20): 3223-28).

[7]. Murray P, Baron E, Jorgensen J, Pfaller M, Yolken R.2003.Manual of Clinical Microbiology.8th edition, PP.719-728.

[8]. Saderi H,Karimi Z, Owlia P, Bahar M.A, Rad, S.M.2008. Phenotypic Detection of Metallo-β - lactamase Producing Pseudomonas aeruginosa Strains Isolated from Burned Patients. Iran. J. Pathol.,3: 20-24.

[9]. Manoharan A, Chatterjee S, Mathai D (2010). Detection and characterization of metallo beta lactamases producing Pseudomonas aeruginosa. Ind. J. Med. Microbiol. 28:241-244.

[10]. Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A, Umadevi S (2013).Detection of metallo-beta-lactamase producing Pseudomonas aeruginosa in intensive care units. Aust. Med. J. 6(12):686-693

[11]. Qu TT, Zhang JL, Wang J, Tao J, Yu YS, Chen YG, Zhou JY, Li LJ (2009). Evaluation of Phenotypic Tests for Detection of Metallo-β-Lactamase- Producing Pseudomonas aeruginosa Strains in China. J.Clin. Micro. 47(4): 1136- 1142.

[12]. Angelescu, M. and Apostol, A. (2001). Cefepime (mexipime), large spectrum 4th generation cephalosporin, resistant to betalactamases. Chirurgia (Bucur). Nov-Dec. 96(6): 547-52 (Abstract).

[13]. Akalin, H.E; (1999). The hole of β-lactam / β -Lactamase inhibitor in the management of mixed infections. J.Antimicrobial. Agents. Chemoth .112 supp 111: 15-20.

[14]. Pagani, L.; Migliavacca, R.; Pallecchi, L.; Matti, C., and Giacobone, E. (2002). Emerging Extended-Spectrum B-Lactamases in Proteus mirabilis, Journal of Clinical Microbiology, Apr. p. 1549-1552.

[15]. Mohanty, S.; Singhal, R.; Sood, S.; Dhawan, B.; Das, B.K. and Kapil, A. (2005). Comparative in vitro activity of betalactam/beta-lactamase inhibitor combinations against Gram negative bacteria. Indian J MED Res 122. pp: 425-428.

[16]. Miller, L.A.; Ratnam, K. and Payane, D.J.(2001). β-lactamase – inhibitor combinations in the 21st century: currentagents and new developments. Curr Opin Pharmacol, 1 : 451-8.

[17]. Al Sahli, A.A. and Abdulkhair, W.M. (2011). Inhibition of beta-lactamase enzyme of Pseudomonas aeruginosa by clavulanic acid of Rumex vesicarius L. African Journal of Agricultural Research Vol. 6(12), pp. 2908-2915.

[18]. Collee , J. G. ; Fraser , A. G. ; Marmion , B. P. and Simmons , A. (1996) . Mackie and McCartney practical medical microbiology . 14th ed. Churchill Livingston . P.173-174 .

[19]. Cruickshank, R.; Duguid, J. R.; Marion, B. P. and Swain, R. H. (1975). The practice of medical microbiology. 12thed. vol. 2. Churchil Livingston, U.K.

[20]. Andrew, G. F.; Gerald, J. C.; Barrie, P. M. and Anthony, S. (1996). Practical medical microbiology. 4th ed. New York- London.

[21]. Holt, J.G.; N.R. Krieg; P.H.A. Sneath; J.T. Staley; and S.T. Williams. 1994. Bergy's Manual of Determinative Bacteriology. 9th.ed. Williams &Wilkins, USA. P.532-551.

[22]. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd.ed. Lippinocott Williams &Wilkins, USA. P.555-565.

[23]. Branson D. "Methods in clinical bacteriology manual of tests and procedures". Springfield. Illinois. USA. 1972.

[24]. Holt J.G, Krieg N.R, Sneath PHA, Staley J.T, Williams S.T. "Bergey,s manual of determinable bacteriology" 9th ed . William and Wilkins, Baltimore. 1994.

[25]. Clinical and Laboratory Standards Institute (CLSI). (2013). Performance Standards for Antimicrobial Susceptibility Testing; twenty- second information supplement; CLSI Document M100-S22. Clinical and Laboratory Standards Institute: Wayne, PA, USA.

[26]. Who .(1978). Techniques for the detection of β-Lactamase producing strains of Neisseria gonorrhoae. 616: 137-143.

[27]. Bhalerao, D.S. ; Roushani, S. ; Kinikar, A.G. and Akhter, I. (2010). Study of Metallo-betalactamase producing Pseudomonas aeruginosa in Pravara Rural Hospital. Pravara Med Rev ;pp 1-5.

[28]. Jarlier , V. ; Nicolas, M. ; Fournier, G. and Philippon, A. (1988). Extended broud-spectrum β-Lactamases conferring transferable resistance to newer β-Lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns . Rev. Infect. Dis. 10(4):867-878.

[29]. Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. (2002). Baily and scott's diagnostic microbiology. 11th ed. Mosby, Inc. St. Louis. USA.

[30]. Brooks, G.; Carroll, K.C.; Butel, J.S. and Morse, S.A. (2007). Staphylococcus. In: Medical microbiology. 24th Ed. 2007. McGraw Hill. Pp 224-230.

[31]. Hashim, S. T. (2005). Isolation and diagnosis of burn contaminated bacteria and study the effect of fat extract on their growth. M.C.S., thesis. College of science. Al- Mustansiriya University.

[32]. Baho, S. I. S. (2006). Genetic study on the locally isolated Pseudomonas aeruginosa and ability in lectin production. M.S.C. thesis, College of science, Al-Nahrain University.

[33]. Ali, N. A. (2009). Study the effect of Allium cepa and Ricinus communis and Vitis vinifera extracts on colony factors activity in bacterial species isolated from otitis media patient. M.S.C., thesis. College of science. Al- Mustansiriya University.

[34]. Atlas, R. M. ; Parks, L. C. and Brown, A. E. (1995) . Laboratory manual of experimental microbiology. 1st ed. Mosby, Inc, USA.
[35]. Al- Hilli, F. A. M. (2000). Study of antibacterial effect of leaves extracts from Callistemon citrinus on P. aeruginosa isolated from patients. M.S.C., thesis. College of science. Al-Mustansiriya University.
[36]. Al-Shaibani, I. N. (2004). Taxonomical study of species belonged to Pseudomonads isolated from Baghdad hospitals and effect of some agents. D.Ph., thesis. College of science. Al-Mustansiriya University.
[37]. Al- Gherawi, R. S. (2009). Effect of Cinnamomum zeylanicum Bark and Apium graveolens L. Seed on the antibiotic resistant bacteria isolated from UTI female patients (in vitro). M.S.C., thesis. College of science. Al- Mustansiriya University.
[38]. National Committee For Clinical Laboratory Standareds (2002). Perfomance Standared For Antibiotic Susceptibility Testing NCCLS. Villanova P.A.
[39]. Abdullah, R.M. ; Samaan, S.F. and ALShwaikh, A.M.(2010). Study the effect of antibiotic combination of beta-lactam and aminoglycoside with another group of antibiotics and their synergism effect . Journal of Arab Board of Health Specializations , Vol.11 , No 1.
[40]. Sotto, A.; de Boever, C. M.; Fabbro-Peray, P.; Gouby, A.; Sirot, D. and Joudan, J. (2001). Risk factors for antibiotic resistant Escherichia coli isolated from hospitalized patients with Urinary Tract Infection: a Prospective study. J. Clinical Microbiol. 39: 438-444.
[41]. Qasim, K.W. (2006). Effect of some chemical and physical factors on Pseudomonas aeroginosa membrane permeability. M.S.C. thesis . College of Sciences . Baghdad University.
[42]. Al-Mashhadani KA. A Study on Diagnosis and Pathogenesis of Pseudomonas aeruginosa Isolated from Different Sources In Mosul City. Ph. D. Thesis. Mosul UniversityCollege of Science. 2004.
[43]. Livermore DM. β–Lactamases- mediated resistance and opportunistic for it's control. J. Antimicrob. Chemother. 41, Sappl. D.25-41. 1998.
[44]. Mirsalehian A, Feizabadi M, Nakhjavani FA, Jabalameli F, Goli H, Kalantari N (2010). Detection of VEB-1, OXA-10 and PER-1 genotypes in extended-spectrum beta-lactamase-producing Pseudomonas aeruginosa strains isolated from burn patients. Burns, 36: 70-74.
[45]. Lim KT, Yasin RM, Yeo CC, Puthucheary SD, Balan G, Maning N, Wahab ZA, Ismail N, Tan EA, Mustaffa A, Thong KL (2009). Geneticfingerprinting and antimicrobial susceptibility profiles of Pseudomonas aeroginosa hospital isolates in Malaysia. J. Microbiol. Immunol. Infect., 42: 197-209.
[46]. Ullah F, Malik SA, Ahmed J (2009). Antimicrobial susceptibility and ESBL prevalence in Pseudomonas aeruginosa isolated from burn patients in the North West of Pakistan. Burns, 35(7): 1020-5.
[47]. Woodford N, Zhang J, Kaufmann ME, Yarde S, Tomas Mdel M, Faris C, Vardhan MS, Dawson S, Cotterill SL, Livermore DM (2008). Detection of Pseudomonas aeruginosa isolates producing VEB-type extended-spectrum β-lactamases in the United Kingdom. J. Antimicrob. Chemother., 62: 1265-68.
[48]. Aggarwal R, Chaudhary U, Bala K (2008). Detection of extendedspectrum β- lactamase in Pseudomonas aeruginosa. Indian. J. Pathol. Microbiol., 51: 222-224.

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