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Investigation of gene resistance on bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumonia* isolates from patient with burn and wound infection to some antibiotics

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Abstract : Objectives: the aim of this study identify the causing agent of infection in burn patient and the sensitivity pattern of some antibiotic. **Methods:** A total of (40) clinical isolates from patient with burn and wound infection,(9) isolate was gram positive bacteria, (15) isolate identified as *Pseudomonas aeruginosa*, ,(11) isolate *Klebsiella pneumonia* and (5) isolate sterile. **Results:** The results showed that percent of infection in female was %67 more than the male %33 and the range of age (20-29) year more than the other years to exposure of infection , and the results showed that *Pseudomonas aeruginosa* was sensitive to imipenem, the percent of resistant was%20,while ceftazidme the percent of resistant was %93. also *Klebsiella pneumonia* was sensitive to imipenem,the percent of resistant %18, while cefepime the percent of resistant was %100. The MICs for *K.pneumoniae* isolates were (≤ 64 , 64 , 64-256, 32-512, 64-512) µg/ml for (Imipenem, Ciprofloxacin, Amikacin, Ceftazidime and Ceftriaxone) respectively, while the MICs for *Ps. aeruginosa* were (≤16, 64, ≤512, 4-512, 64-512) µg/ml for (Imipenem, Ciprofloxacin, Amikacin, Ceftazidime and Ceftriaxone) respectively.**Conclusions:** All isolates gave negative results for detection of Metallo-β-lactamase by EDTA method. The genotypic detection by PCR showed that (3) isolates of *K.pneumoniae*and (5) isolates of *Ps. aeruginosa* had bla_{CTX-M} genotype only while all isolates were negative for bla_{SHV} and bla_{TEM} genotype.

Key Words : Gene resistance, antibiotics sensitivity, burn and wound infection, genotypic detection by PCR, exposure of infection.

Introductions

P.aeruginosa prefers growth in moist environments, as a reflection of its origins in soil and water [21]. *P. aeruginosa*produces many types of soluble pigments of which ,pyocyanin and pyoverdin are the most common type . The latter is produced abundantly in media of low-iron content, and it involves in iron metabolism. Pyocyanin refers to "blue pus" which is a characteristic of supportive infections caused by *P.aeruginosa*[3]. Other produced pigments are pyorubin (red), pyomelanin (brown) and pyoverdin (yellow/green)[2] Infection with *P. aeruginosa*can lead to urinary tract infections , sepsis (blood stream infection), pneumonia, meningitis, and many other medical problems beside It colonizes the lungs in patients with cystic fibrosis (CF) and contributes to the chronic progressive pulmonary disease[3]. This species shows a remarkable capacity to resist different antibiotics intrinsically due to constitutive expression of β-lactamases and efflux pumps combined with low permeability of the outer membrane[4,5]. Other mechanism involved in antibiotic resistance in this bacteria include production of modifying enzymes which render the antibiotic inactive such like β-lactamases or enzymes inactivating aminoglycosides or modifying their target . Production of β -lactamases is one of the main mechanisms of resistance in this bacteria toward β-lactams group [6]and numerous β-lactamases encoded

by conjugative transferable plasmid or chromosomally mediated have been reported in *P.aeruginosa*[4] .Since that , infections caused by *P. aeruginosa*are difficult to treat as the majority of isolates exhibit varying degrees of β-lactamase mediated resistance to most of the β-lactam antibiotics beside its resistance to wide range of other antibiotics[7]. In a research carried out by[8]. they illustrated that high levels of resistance to various aminoglycosides in this bacteria was encoded by conjugative plasmid responsible for ribosomal protection by methylation 16S rRNA beside their ability to produce β-lactemase enzyme[9]. Many recent studies reported that the prevalence of 16S rRNAmethylation in *P.aeruginosa*for*RmtA* gene according to Yamane *et al*. (2004)[10]. found to be 29% ,while it reached 15.4% to *RmtD* in Doi*et al*.(2008)[11]., and the percentage for *RmtD* increased to reach 36.6% according to[8]. in their study in Brazil .In South Korea the prevalence rate of *ArmA* reached 14% and consider the first report for occurrence of this type of resistance in *P.aeruginosa*[12].It could be said that the rates of antibiotic resistance in *P.aeruginosa* are increasing worldwide and multidrug-resistant phenotype in this bacteria could be mediated by several mechanisms of resistance and can pose a major threat to life and are often difficult and too expensive to be treated [13] .

Experimental

Samples collection

Wound and burn sample were collected from (40) patients undergo from burn hospital in medical city and Alemam-Ali hospital in Baghdad during the period between November 2012 to December 2012. (5) sample are sterile and (9) sample contain gram positive, while (30) sample contain (15) smear to *P.aeruginosa* and (11) smear to *Klebsiella pneumonia* .

Characterization and Identification of the Isolates

The collected samples were streaked on freshly prepared nutrient agar plates and incubated aerobically and anaerobically at 37^{o}C for 24 hours. Bacterial colonies differing in size, shape and colour were selected from the different plates and further subcultured on nutrient agar by the streak plate technique and incubated at 37^{o}C for 24 hours after which, were maintained in agar slants for further characterization and identification. The bacterial isolates were characterized based on colonial and cell morphology, growth on differential/selective media and biochemical tests which include Gram's reaction, indole tests, methyl red, voges-proskauer, citrate utilization, motility, endospore, utilization of carbohydrates such as glucose, sucrose, mannitol, lactose and fructose, oxidase, catalase, coagulase and starch hydrolysis test[14]. The bacterial isolates were identified by comparing their characteristics with those of known taxonomy using the schemes of [15].

Bacterial Diagnosis

Morphological Examination

Primary diagnostic based on morphological characteristic of the colonies including colony shape, colony texture, color and edges were studied depending on bacterial growth on MacConkey agar and blood agar [16] . Final identification for the isolates was dependent on the results of api 20E. The api 20E system is a standardized and miniaturized version of conventional procedures for the identification of Enterobacteriaceae and other Gram-negative bacteria[15].

Microscopic Examination

One isolated colony was transported to a microscopic slide, fixed then stained with Gram stain .Gram reaction, cell shape and arrangement were observed .The results were compared with [16].

Antimicrobial Susceptibility Test

All isolates of *P.aeruginosa*&*k. pneumoniae* were tested of sensitivity to 10 of antibiotic are Ceftriaxone (CRO 30µg) Ceftazidime (CAZ 30µg) (IPM10 µg) ImipenemCefepime (FEP 30µg) Amikacin (AK 30µg) (GM 10 µg) Gentamicin (CIP 5µg)Ciprofloxacin Piperacillin(PIP100µg) Aztreonem (AT 30µg) (Tcc 75µg) Ticarcillin by disk method on Mueller-Hinton agar.All isolates were tested for antimicrobial susceptibility depending on the inhibition zone [17].

Determination of the Minimum Inhibitory Concentration (**MIC):**Minimum inhibitory concentration (MIC) was determined for all isolates according to the CLSI (2011) criteria by a standard agar dilution methodforthe antibiotics(Imipeneme, Ciprofloxacin, Amikacin, Ceftazidime and Ceftriaxone)using Mueller-Hinton agar.

Screening for Metallo-β-lactamase: This test carried out by EDTA method as describedin[18] .

Preparation of DNA Template:

DNA template was prepared as described by [19].

Polymerase Chain Reactions: PCR reactions were carried out by using Green Master Mix (Promega/USA)and specific primer for each gene(Alpha DNA/Canada), the addition of each primer to the PCR mixture. Table(1) .PCR mixture consist of (12.5) µl green master mix, (5)µl DNA template, the addition of each specific primers with concentration (10) pmole/µl Table(1), finally this mixture was completed to (25) µl by adding nuclease free distteled water. The reactions carried out in specific condition Table(2) by Polymerase Chain Reaction thermal cycler(TechNet – 500/USA). PCR products were electrophoresed using Electrophoresis power supply(Cleaver scientific /Taiwan) and the size of product was measured by 100bp DNA molecular ladder(KAPA/south Africa).

Table(1):Sequences and additions volumes of each primer to the PCRMixture.

Primer	Sequence 5'— 3'	Prod. Size (bp)	Ref.	Addition vol.(µl)
bla_{CTX-M}**F**	5'-CGCTTTGCGATGTGCAG-3'	550	4	2
bla_{CTX-M}**R**	5'-ACCGCGATATCGTTGGT-3'			2
bla_{SHV} **-F**	5'-AAGATCCACTATCGCCAGCAG-3'		5	1.5
bla_{SHV}**-R**	5'-ATTCAGTTCCGTTTCCCAGCGG-3'	200		1.5
bla_{TEM}**-F**	5'-GAGTATTCAACATTTCCGTGTC-3'	800	5	1
bla_{TEM} **-R**	5'-TAATCAGAGGCACCTATCTC-3'			1

Table(2): Uniplex PCR reaction condition of bla_{SHV} ,bla_{TEM}, bla_{CTX-M}

Gene	Initial denaturation	No. of cycles	Denaturation	Primer annealing	Extension	Final extension
bla_{SHV}	95Cº / 5 min	35	95Cº / 1min	59ºC / 1min	72Cº / 1 min	72Cº / 10 min
bla_{TEM}	95Cº / 5 min	35	94Cº / 30 second	55ºC / 30 second	72Cº / 1 min	72Cº / 6 min
bla_{CTX-M}	94 Cº / 5 min	35	95Cº /30 second	55ºC / 1min	72Cº / 1 min	72Cº / 6 min

Results

Wound and burn sample were collected from 40 patients then streaked on freshly prepared nutrient agar,blood agar and MacConkey agar,5 isolate were sterile only 26 isolate from 35 isolate were showed culture on MacConkey agar and 9 isolate were not showed culture on MacConkey agar contain gram positive bacteria and 15 isolate contain*P.aeruginosa* while 11 isolate was*Klebsiellapneumonia* are shown in fig (1).

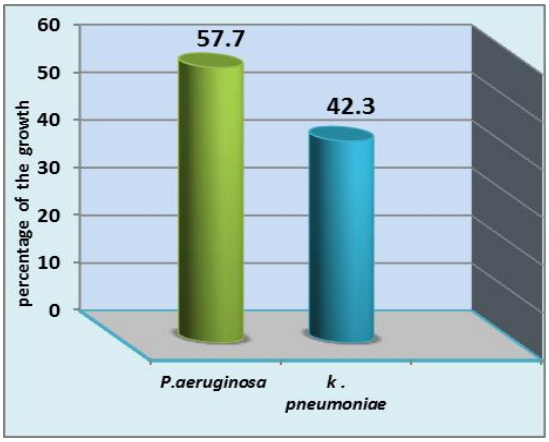
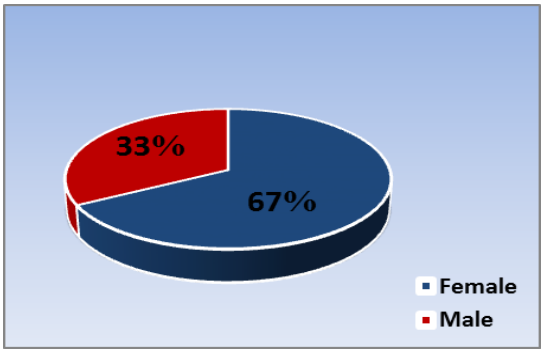


Figure :(1) bacteria on the culture mediaof the growth percentage

The result of this study coincide with the results of (*dilnawaz et al.*,2004) in bakistan showed found of *P.aeruginosa* with percent of 30% then *staph aureus 28%*,*Klebsiellapneumonia* 16%,proteus ssp 14%,*E.coli*6% and finaly*staphepidermidis* 6%.In this study,the percent of infection in female to male were 2:1 (16 burn infection in female 66%)while (10 burn in fection in male 33%)[20] . are shown in fig.(2).



Figure(2): percentage of infection male and female

The result of this study coincide with the result of [20] in Pakistan, this study showed found *P.aeruginosa* 30% , *Staph. aureus* 28% ,*Staph.epidermidis* 6%,*Klebsiella pneumonia* 16%,Proteus ssp 14% and finally *E.coli* 6% .Percent of infection in female to male were 2:1 (16 burn infection in female 66.6%),while(10 burn infection in male 33.3%) are shown in fig.(2).

The result coincide with the result of [21]. in al - Musal university ,the study found the percent of burn infection was 30%.study in Iran showed found percent of burn infection in female 64% ,while in male 35%

This study showed that rang of age between (20-29)year more response to burn infection 40% may be because of infection in working or because of false using for heating energy source then the rang of age between (10-19)year. The infection in children may be because of exposure to heating liquid such as tea.

All isolates of *P.aeruginosa* & *k. pneumonia* were tested of sensisitivity to 10 of antibiotic are Ceftriaxone (CRO 30µg) Ceftazidime (CAZ 30µg) (IPM10 µg) Imipenem Cefepime (FEP 30µg) Amikacin (AK 30µg) (GM 10 µg) Gentamicin (CIP 5µg)Ciprofloxacin Piperacillin(PIP100µg) Aztreonem (AT 30µg) (Tcc 75µg) Ticarcillin by disk method on Mueller-Hinton agar.All isolates were tested for antimicrobial susceptibility depending on the inhibition zone (16) are shown in fig.(3).

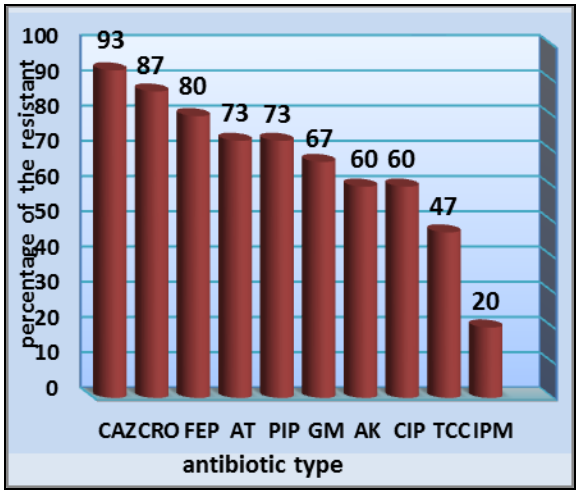


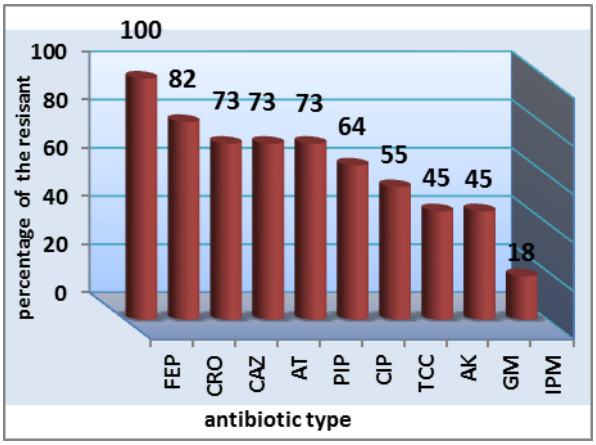
Figure (3):percentage of *P. aeruginos*a bacteria isolate which resistant to antibiotic

Imipenem was more effective against to *P.aeruginosa* with percent of resistant was 20%,the percent of resistant Ticarcillin was 47%, percent of resistant to Ciprofloxacillin was 60%,Amikacin was 60%,Gentamycillin was 67%,piperacillin &Aztreonem was 73% for each one, Cefeoime was 80%,Ceftazidime was 87% and Ceftriaxone was 93%.

This result Conicide with results of study in Tywan in this study isolate of *P.aeruginosa* showed percent of resistant to Gentamicin was 69.4% , to Cefepime Ciprofloxacin was 61.2% for each one , Ceftazidime was 38.8% , Aztreonam was 42.9% and f finally to Meropenem was 42.9% [22] .

The resistant of *P.aeruginosa* to Ceftrinxone , Ceftazidime and Cefepimemay be because of production ESBLs enzyme and presence of the gene that responsible for production the enzyme in resistant isolate.

While for *K.pneumonia* are shown in fig.(4).



Figure(4):percentage of *K. pneumoniea* bacteria isolate which resistant to antibiotic

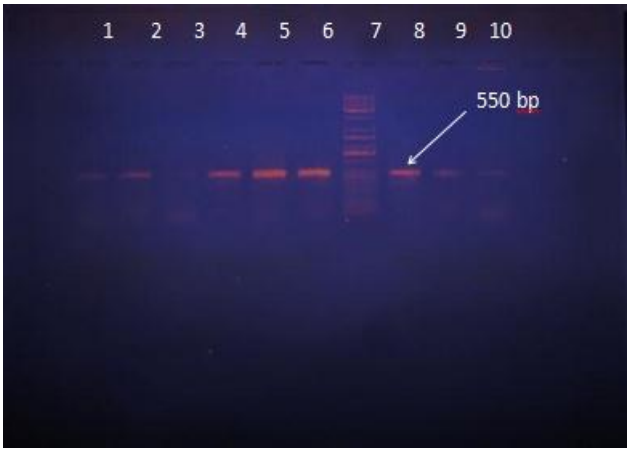
The percent of resistant to Imipenem was 18%,Gentamycin was 45%,Amikacin was 45%,Ticarcillin was 55%,Pipercillin,Aztreonem and Ceftazidime was 73% for each one, Ciprofloxacillin was 64%Ceftriaxone was 82% and finally to Cefepime was 100%.resistant of *K.pneumonia* to Cephalosporin's may be because of production Beta-lactamase enzyme [28] .

This system consider as a rapid and easy methods for identification of the bacteria. by using this system, it was confirmed that these isolates were *P.aeruginosa*.MICs results for*K.pneumonia* isolates were(≤ 64 , 64 , 64-256 , 32-512, 64-512) µg/ml for (Imipeneme, Ciprofloxacin, Amikacin, Ceftazidime and Ceftriaxone) respectively, the MICs for *Ps. aeruginosa* were (≤16, 64, ≤512, 4-512, 64-512)µg/ml for (Imipeneme, Ciprofloxacin, Amikacin, Ceftazidime and Ceftriaxone) respectively. Table(3). While all isolates showed

negative results for detection of Metallo-β-lactamase by EDTA method. PCR in this study gave negative results for both of blaSHV and blaTEM genotype, while (3) isolates of *K.pneumoniae* and (5) isolates of Ps. Aeruginosa showed positive results forblaCTX-M genotypeFigure (3).

Table(3):MICs results for *K.pneumoniae*&*P.aeruginosa*.

Isolates.	IMP µg/ml	CAZ µg/ml	CTX µg/ml	CIP µg/ml	AK µg/ml
K.1	16	32	256	64	256
K.2	16	4	64	-	64
K.3	16	32	256	64	-
K	16	512	512	64	-
K	-	-	-	64	-
K.23	16	64	64	-	-
K.50	-	32	-	64	512
K.210	64	256	512	64	512
Ps.*	-	64	128	64	256
Ps.6	-	64	64	64	512
Ps.10	-	64	16	-	-
Ps.11	-	512	256	-	512
Ps.12	16	4	512	-	-
Ps.14	8	4	128	-	64
Ps.15	-	256	64	-	64
Ps.16	-	256	256	64	128
Ps.18	-	-	512	64	512
Ps.35	-	512	128	64	512
Ps.36	-	512	512	-	512
Ps.47	-	256	128	-	-



Figure(3):Gel electrophoresis (1% agarose, 7V/cmfor 45 min)for *bla*$_{CTX-M}$gene.Lanes1 , 2 , 3 , 4 , 5 , 6) :*Ps.aeruginosa* isolates (47, 36, 35, 15, (12,11) respectively. Lane-7:100bp DNA ladder. Lanes (8,9,10): *K.pneumoniae* isolates (1,3)respectively.

Discussions

Bacteria that produce ESβL are frequently resistantto other antimicrobial agents, such as aminoglycosides,tetracycline, and trimethoprim-sulfamethoxazole, as manyof these additional resistance genes are encoded on the same ESβL-associated plasmid. Fluoroquinolone resistance,which is also frequently associated with ESβL production,is usually chromosomally encoded, unlike the other co-resistances, however,

plasmid-mediated quinolone resistance has been discovered recently Risberg, K. (2010).Al-Kaabi, M.H.A. (2011) noticed that the boiling method[23] was better in illustrating the results as compared with using plasmid template for detection ESβLsgenes. Boiling method also good method for verifying the results and it is more common in routine work. Phenotypic methods are not able to distinguish between the specific enzymes responsible for ESβL production (SHV, TEM, and CTX-M types). Several researches or reference laboratories use genotypic methods for the identification of the specific gene responsible for the production of the ESβLs, which have the additional ability to detect low-level resistance (i.e, can be missed by phenotypic methods). Furthermore, molecular assays also have the potential to be done directly on clinical specimens without culturing the bacteria, with subsequent reduction of detection time Farkosh, M.S.(2007)[24]. PCR technique represents the easiest and most common molecular method used to detect the presence of a β-lactamase belonging to a family of enzymes. This technique is very sensitive, easy to perform, specific for gene families and very efficient compared with the other methods Bradford (2001)[25].Themembers of gram negative bacteria can acquire resistance to extended spectrum beta-lactams by a different mechanisms; the most important one being the plasmid encoded extended spectrum β-lactamase (ESβL) and AmpC beta lactamases[26] .Recently, bacterial pathogens are more complicated than those isolated before a decade or two ago .They don't only have new resistance mechanisms represented by boarder extended spectrum β- lactamase (ESβLs), but such isolates also produce multiple β-lactamases causing serious therapeutic problem in many parts of the world[27].

References

1. Eldere, J.V. Multicentre surveillance of Pseudomonas aeruginosasusceptibility patterns in nosocomial infections.J Antimicrob. Chemother. 2003; 51:347–352.
2. Brooks, G.F.; Butel, J.S.; Carroll, K.C. and Morse, S.A. Jawetz, Melnick&Adelberg's medical microbiology. 24th ed. McGraw-Hill, New York. U.S.A, 2007.
3. Goldman, E. and Green, L.H. Practical Handbook of Microbiology. 2nd edition . Taylor & Francis Group. New York. USA. 2009;230-244.
4. Livermore, D. M. and Woodford, N. The β-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends in Microbiol14.2006 ; (9): 413-420.
5. Ashraf S. Hassan . The Antibacterial Activity of Dimethyl Sulfoxide (DMSO) with and without of Some Ligand Complexes of the Transitional Metal Ions of Ethyl Coumarin against Bacteria Isolate from Burn and Wound Infection . Journal of Natural Sciences Research 2014 , Vol.4, No.19, pp: 106 .
6. Mesaros, N.; Nordmann, P.; Ple´siat, P.; Roussel-Delvallez, M.; Eldere, J.V.; Glupczynski, Y.; Laethem, Y.V.; Jacobs, F.; Lebecque, P.; Malfroot, A.; Tulkens, P.M. andBambeke, F.V. Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium. J.Clin. Microbiol. Infect. 2007;13: 560–578.
7. Upadhyay, S.; RanjanSen, M.; Bhattacharjee, A. Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosaexpressing AmpC beta-lactamase enzyme. J. Infect. Dev. Ctries. 2010 ; 4(4):239-242.
8. Neves, P.R.; Mamizuka, E.M.; Levy ,C.E. and Lincopan, N. Multidrug-resistant Pseudomonas aeruginosa: an endemic problem in Brazil.Bras. J. Patol. Med. Lab. 2011; 4(47): 409-420.
9. Mansour, W.; Dahmen, S.; Poirel, L.; Charfi, K.; Bettaieb, D.; Boujaafar, N. and Bouallegue, O. Emergence of SHV-2a extended-spectrum β-lactamases in clinical isolates of Pseudomonas aeruginosain a univer-sity hospital in Tunisia. Microb. Drug Resist. 2009;15: 295–301.
10. Yamane, K.; Doi, Y.; Yokoyama, K.; Yagi, T.; Kurokawa, H.; Shibata, N.; Shibayama, K.; Kato, H., and Arakawa,Y. Genetic Environments of the rmtAGene in Pseudomonas aeruginosaClinical Isolates.Antimicrob.Agent.Chemother. 2004;6(48): 2069-2074.
11. Doi, Y.; Garcia, D.O.; Adams, J. and Paterson, D.L. RmtD 16S RNA Methylase in Epidemiologically Unrelated SPM-1-Producing Pseudomonas aeruginosaIsolates from Brazil. J. Antimicrob. Agent. Chemother. 2008;4(52): 1587-1588.
12. Gurung, M.; Moon, D.C.; Tamang, M.D.; Kim, J.; Lee, Y.C.; Seol, Y.S.; Taek, D. and Lee, C.J.C. Emergence of 16S rRNAmethylase gene armA and cocarriage of blaIMP-1 in Pseudomonas aeruginosa isolates from South Korea. J. Diagnos. Microbiol. Infect. Dis. 2010;4(68): 4468-470.
13. Hirsch, E.B. and Tam, V.H. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Exp. Rev. Anti-infect. Therapy. 2010; 4(10):441-451.
14. Brown, A.E. Benson's Microbiological Application .9th .ed. The McGraw-Hill Companies, USA , 2005.

15. Atlas, R.M.; Brown, A.E. and Parks, L.C. Laboratory manual of experimtal microbiology. 1st ed. Mosby, st. Louis, U.S.A,1995.
16. Holt, J.G.; Kreieg, N.R.; Sneath, P.H.; Staley, J.T. and Williams, S.T. Bergey`s Manual of Determinative Bcteriology. 9th ed. Wikins. 1994 , pp: 1063.
17. CLSI, (Clinical and Laboratory Standards Institute) Performance standard for antimicrobial susceptibility testing; Twenty-First informational supplement. M100-S21. 2011;31(1).
18. Sederi,H.; Karimi,Z.;Owlia,P.; Bahar,M.A.; Rad,S.M.Phenotypic detection of metallo-beta-lactamase producing Psudomonas aeruginosa isolate from burned patients. Iran. J. Pathol. 2008; 3(1):20-24.
19. 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. & White, T.J. Diagnostic Molecular Microbilogy. 9th ed. American Society for Microbiology. Washington, DC, 1993.
20. Dilnawaz S ., S. Zaidi DI, A. H., Khurram S. , Munima S. , Baqir S. N., M.R. Shaikh and M. Harriss S., (Incidence and resistance pattern of bacteria associated with burn wound sepsis) Pakistan Journal of Pharmacology Vol.21, No.2, July 2004, pp.39-47.
21. Haitham M. Al-Habib, Asmaa Z. Al-Gerir, Ansam M. Hamdoon Profile of Pseudomonas aeruginosa in burn infection and their antibiogram study(Ann. Coll. Med. Mosul 2011; 37 (1 & 2): 57-65.a.
22. Risberg, K.. Aminoglycoside resistance mechanisms in Enterobacteriaceae. Department of Pharmacy. Faculty of Medicine. University of Tromsø. Norway, 2010.
23. Al-Kaabi, M.H.A. Detection of TEM and extended spectrum β-lactamase enzymes produced by some Gram negative bacteria using polymerase chain reaction. M. Sc. Thesis. College of Science .Al-Mustansiryah University, 2011.
24. Farkosh, M.S. Extended-Spectrum betalactamase Producing Gram Negative Bacilli. http://nosoweb.org/infectious diseases/esbl.htm, 2007.
25. Bradford, P. A. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. ClinMicrobiol Rev . 2001; 14: 933-51.
26. Branger, C.; Zamfir, O.; Geoffroy, S.; Laurans, G.; Arlet, G.; Thien, H.V.; Gouriou, S.; Picard, B. and Denamur, E. Genetic Background of Escherichia coli and Extendedspectrum β-Lactamase Type. Emerg. Infect. Dis. 2005; 1(11):54-61.
27. Sharma, J.; Ray, P. and Sharma, M. Plasmid profile of ESBL producing Gram-negative bacteria and correlation with susceptibility to β-lactam drugs. Indian. J. Pathol. Microbiol. 2010; 1(53): 83-86.
28. Buehlmann M, Bruderer T, Frei R, Widmer AF. Effectiveness of a new decolonisation regimen for eradication of extended-spectrum β-lactamase-producing Enterobacteriaceae. J Hosp Infect 2011; 77: 113–7.

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