Multidrug Resistant Bacterial Strains and their Associated Plasmid Profile

Zahira M.F. El-Sayed[(1)], Ahmad K. Al-Ghamdi[(1)], Esam I. Azhar[(1)], Nahlaa A. Khalifa[(2)], Ahmed M. Ashshi[(3)], and Hani S. Faidaha[(4)]

[1]Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, King Abdulaziz University Jeddah, Saudi Arabia.
[2]Department of Clinical Nutrition, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.
[3]Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University.
[4]Microbiology Department, Faculty of Medicine, Umm Al-Qura University.
drzahira2009@yahoo.com

Abstract: Development and spread of bacterial resistance is usually attributed to the abuse of antibiotics. The emergence of resistance to different antibiotics is a particular problem when treating infections. The study aimed to evaluate plasmid profiles of different bacterial strains which proved to be multi drug resistant. Four strains of isolated *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli), Klebsiella pneumoniae(K. pneumoniae)* and *Pseudomonas aeruginosa (P. aeruginosa)* were tested for their sensitivity to plasmid profile of the four selected isolates. *S. aureus* showed presence of plasmid with molecular weight (23.130 Kbp), *E. coli* showed presence of plasmid with molecular weight between (23.130 Kbp and 9.416 Kbp), *K. pneumoniae* showed presence of plasmid with molecular weight (23.130 Kbp) and for *P. aeruginosa* showed presence of plasmid with molecular weight between (23.130 Kbp and 9.416 Kbp). These results indicated that the resistance to antibiotics for all isolates due to the presence of plasmids in all isolates. In conclusion drug resistance is serious problem over the entire world, the results showed that the resistance to antibiotics for all isolates was due to the presence of plasmids.
[Zahira M.F. El-Sayed, Ahmad K. Al-Ghamdi, Esam I. Azhar, Nahlaa A. Khalifa, Ahmed M. Ashshi and Hani S. Faidaha. **Multidrug Resistant Bacterial Strains and their Associated Plasmid Profile.** *Life Sci J* 2015;12(1):1-9]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 1

Keywords: bacterial resistance, antibiotics, plasmid, electrophoregram.

1. Introduction

Development and spread of bacterial resistance is usually attributed to the abuse of antibiotics. The use of these agents for any infection in any dose and over any time period, forces microbes to adapt or die **(Al-Bayati, 2008).**

The microbes which survive are those that carry genes for resistance to antimicrobial agents **(Nickerson** et al., 2009). However, antibiotic resistance is not a single phenomenon, and many resistance mechanisms have been identified and analyzed for most clinical pathogens and almost all antibiotics for clinical use **(Hall et al, 2003 &Jarvis et al., 2007).**

Antibiotic resistance probably has different origins and must be as ancient as antibiotic synthesis **(DeLeo & Chambers, 2009: Aiello et al., 2006 and Alarn et al., 1994).** Resistance genes pre-existed in nature, in soil and water, and their presence was probably related to the production of antibacterial agents synthesized naturally in environment by saprophytic organisms **(Hughes et al., 2002 &Jones, 2003).** Global epidemiology of

Haung and Rybak (2005) showed that, the cefepime has broad spectrum activity against gram negative and gram positive organisms. The authors demonstrated that the combination of cefepime with vancomycin, linezolid or quinupristin dalfopristin had an improved or enhanced effect against methicillin resistance *Staphylococcus aureus*. Whether resistance is intrinsic or acquired bacteria share pathways to induce resistance to antimicrobial agents **(Bonfiglio et al., 1998).** These pathways include enzymatic degeneration or modification of antimicrobial agents, decreased uptake or accumulation of antimicrobial agents, altered antimicrobial target, circumvention of consequences of antimicrobial action (**Cirioni et al., 2007)**' uncoupling of antimicrobial agents–target interactions and subsequent effects on bacterial metabolism or any combination of the above mechanisms **(Jeljaszewicz et al., 2000).**

Extensive resistance of antibiotic has been developed in gram-negative bacteria due to both innate resistance in some species and the fact that they are highly adapted at acquiring antibiotic resistant determinants from each other **(Waterer and Wunderink, 2001 and Klein et al., 2007).**

It was noted that some strains of *E. coli* and *K. pneumoniae* acquired plasmid-mediated resistance to third generation cephalosporin that can spread from species to species and are usually blocked by lactamase inhibitors (**de Lencastre et al., 2007**).

Following the over use of the expanded spectrum cephalosporins, sever outbreaks by expanded spectrum ß-lactamases producing *E. coli* and *K. pneumoniae* have been reported **(Waldron &Lindsy, 2006).** High resistance of trimethoprim in members of the family Enterobacteriaceae is most often caused by acquired plasmid that encodes dihydrofolate reductase (DHFR) less sensitive than the chromosomal enzyme to inhibition by trimethoprim (**Hamouda, 2000 and Klevens, 2007).**

Widespread quinolone resistance has been observed in clinical isolates of multidrug –resistant bacteria such as methicillin- resistant *S. aureus* and coagulase–negative *Staphylococcus*, this may be attributed to mutation in the gyIA genes encoding the A subunits of gyrase and topo IV, and thus the effectiveness of newer quinolones against these pathogens will depend on their ability to overcome existing resistance due to specific point mutation and inhibit targets simultaneously, thereby lowering the likehood of stepwise resistance development **(Roychouldhury et al., 2001 and El Behedy et al., 2000).**

Plasmid typing or plasmid fingerprinting, is a relatively new addition to the typing systems used in clinical microbiology. Plasmid DNA content is a unique and relatively stable characteristic of most bacteria, and such it has gained importance as an epidemiologic tool. Preparing plasmid is a relatively simple, inexpensive procedure and the same methodology applied to most microorganisms. In many cases plasmid profiles offer a higher level **(Domenico et al., 1992).**

Plasmid from one strain can be distinguished from those of another on basis of their molecular sizes. Plasmid size is expressed as the number of kilo base pairs of DNA and is determined electrophoretic nitrogen in agarose gels. The genetic information encoded on plasmid DNA can define or influence other typing system used in clinical microbiology, including the biotype, serotype and antibiotic susceptibility of particular strain **(Tompkins, 1985).**

Aim of the work: to detect plasmid profiles of some bacteria strains which proved to be multidrug resistance, also to detect their protein fingerprint to revel the association of their resistance and protein bands.

2. Materials and Methods

This study was carried on four isolated strains from patients admitted to King Abdulaziz University Hospital; the strains were investigated and revealed multidrug resistant to many chosen antibiotics.

All the previous isolated strains were subjected to the following:

1-Antibiotic susceptibility testing: -

Bacterial strains isolated from different infections from immunocompromised patients were tested for their antimicrobial sensitivity by standardized disc diffusion technique according to **(Collee et al., 1989).**

The isolates were tested against 18 antibiotics namely; Imipenem, Cefotaxime, Gentamicin, Velosef, Chloramphenicol, Rifampicin, Streptomycin, Tetracycline, Flummox, Penicillin, Vancomycin, Amoxicillin, Oxacillin, Negram, Duricef, Sxt, Erthromycin, and Ampicillin.

- The four detected multidrug resistant ***S. aureus*, *E. coli, K. pneumoniae* and *P. aeruginosa*** were used for plasmid recognition.

2- Plasmid profile:

Detection of Plasmid DNA in the four tested strains was done according to **(Maniatis et al. 1982).**

A- Isolation of plasmids of the multiple drug resistant strains (Suzuki et al, 1978): - Each strain of all the selected multidrug MRSA strains were grown in 5 ml of Luria broth (L-broth) overnight at 37ºC. The cells were harvested by centrifugation then supernatant was poured off, and the pellet was resuspended in solution 1 (glucose, EDTA and Tris hydrochloride) then mixed by inversion and left in ice for 15 min.

- Solution II (sodium hydroxide and SDS), was added and mixed by vortexing and left on ice for 5 min, then solution III (potassium acetate) was added and vortexing left on ice for 30 min, and centrifuged for 5 min at 4ºC. The supernatant containing plasmids was precipitated by phenol/ chloroform and ethanol at -70 ºC for30 min then centrifuged for 10 min at 4 ºC at full speed (1500rpm). The supernatant was poured off and a tiny amount of precipitate plasmid DNA separated.

- The pellet was re-suspended in Tris EDTA buffer, then plasmid DNA was put in a tube and buffer was added. The samples were loaded to 0.8 % agarose gel and molecular weight markers (λ Hind DNA marker) were run.

3- SDS-PAGE Protein Electrophoresis: (Hartinger et al, 1996)

Agarose gel was prepared according to **(Sambrook et al., 1989),** then samples of plasmid DNA preparations were loading in the well of agarose gel and a 100-v current was run. The gel was transformed for a staining tray containing water mixed with ethidium bromide for at room temperature, then transformed to second tray of water for washing. The gel was visualized by ultra violet ray at 320 nanometer (nm) wave length.

3. Results

Figure (1) Revealed that, the plasmid profile of the four selected isolates Lane *(2)* for ***S. aureus*** showed presence of plasmid with molecular weight

(23.130 Kbp), Lane (3) for ***E. coli*** showed presence of plasmid with molecular weight between (23.130 Kbp and 9.416 Kbp), Lane (4) for ***K. pneumoniae*** showed presence of plasmid with molecular weight (23.130 Kbp) and Lane (*5)* for ***P. aeruginosa*** showed presence of plasmid with molecular weight between (23.130 Kbp and 9.416 Kbp). These results indicated that the resistance to antibiotics for all isolates due to the presence of plasmids in all isolates.

Figure (2) showed protein electrophoregram (scan of protein profiles or finger print of the total cellular protein of the vegetative cell).

Figure (3) shows the protein finger print technique was carried out for the selected isolates to confirm the identification of isolates. Results presented in Figs *(2* and 3) and tables (1, 2 and 3) showed that, in the electrophoregram (scan of protein profiles or finger prints of the total cellular proteins of the vegetative cells of tested isolates No. (19 u, 20 u, 10 u and 30 s) were fractionated in denatured gel by electrophoresis (SDS-PAGE) and the protein bands revealed from 13 to 25 discrete protein bands. The protein profiles isolates (No 19 u. 20 u, 10 u and 30 s) contains 18, 13, 15 and *25,* respectively, as in table (1, 2 and 3) and fig *(2)* which showed the comparison of protein patterns from 4 isolates were similar especially in the high molecular weight parts of the gels except the last isolate no (30 s) which diverse from the other isolates which the highest molecular weight was 126.029. Most of the bands in each isolate could be matched with bands in each of the others.

Table (1) The number of the bands of protein and molecular weight of highest concentration of protein band in relation to (SDS-PAGE).

Bacterial strains	Total No of bands	MW of highest cone of protein
1	**18**	**173.735**
3	**15**	**230.25**
4	**25**	**126.029**

Table (2): Molecular weight of protein products of field collected isolates No *(K. pneumoniae, P. aeruginosa E. coli* and S. *aureus*).

M.W. values;	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
Band1	209	173.735	231.578	230.25	126.029
Band2	124	134.724	178.03	168.091	120.748
Band3	80	119.449	120.748	134.724	116.858
Band 4	491	98.701	96.339	1188	108.541
Band5	348	87.417	82.356	100.501	102.936
Band6		79.093	57.657	85.853	93.455
Band7		72.909	52.789	71.433	87.417
:Band8		61.85	46.227	59.098	82.842
|Band9		55.955	42.227	53.739	76.491
iBand10		52.789	31.409	43.996	71.796
Band11		41.487	26.544	40.451	61.364
Band12		37.033	19.173	34.063	58.875
Band13		33.178	16.372	27.871	56.693
Band14		29.64		19.763	54.395
Band15		26.102		14.898	51.279
Band 16		22.269			47.797
Band17		16.667			45.902
Band18		13.866			42.67
Band19					40.451
Band20					36.736
Band21					34.653
Band22					32.884
Band 23					30.525
Band24					27.576
Band25					17.404

Table **(3): RF-values of protein products of field collected isolates No *(K. pneumoniae, P. aeruginosa, E. coli* and *S. aureus*).**

R.F. values	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
Band 1	0.043	0.088	0.011	0.013	0.16
Band 2	0.164	0.145	0.083	0.096	0.173
Band 3	0.305	0.177	0.173	0.145	0.184
Band 4	0.539	0.239	0.246	0.179	0.209
Band 5	0.722	0.276	0.295	0.233	0.226
Band 6		0.308	0.44	0.282	0.256
Band 7		0.336	0.492	0.344	0.276
Band 8		0.404	0.579	0.427	0.293
Band 9		0.457	0.658	0.481	0.32
Band 10		0.492	0.765	0.605	0.342
Band 11		0.637	0.827	0.65	0.48
Band 12		0.694	0.921	0.731	0.43
Band 13		0.742	0.957	0.81	0.449
Band 14		0.788		0.914	0.474
Band 15		0.833		0.976	0.511
Band 16		0.882			0.556
Band 17		0.953			0.581
Band 18		0.989			0.622
Band 19					0.65
Band 20					0.697
Band 21					0.724
Band 22					0.746
Band 23					0.776
Band 24					0.814
Band 25					0.944

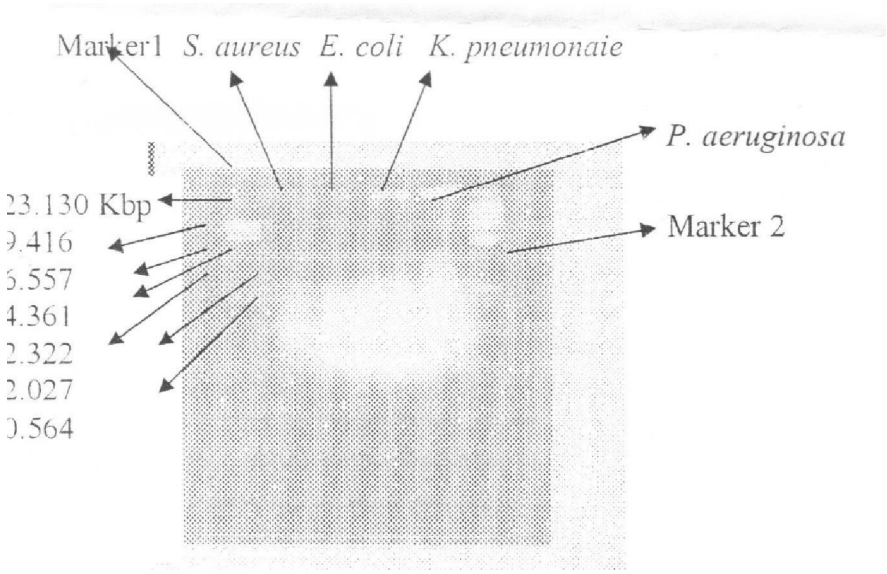
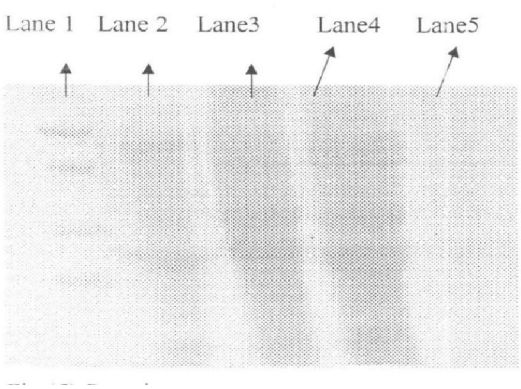


Figure (1): the plasmid profile of the four selected isolates.



Lane 1 = marker
Lane 2 = 19 (u) *K. pneumoniae*
Lane 3 = 20(u) *p. aeruginosa*
Lane 4 = 10 (u) *E. coli*
Lane 5 = 30(s) *S. aureus*.

Figure (2): Protein electrophoregram.

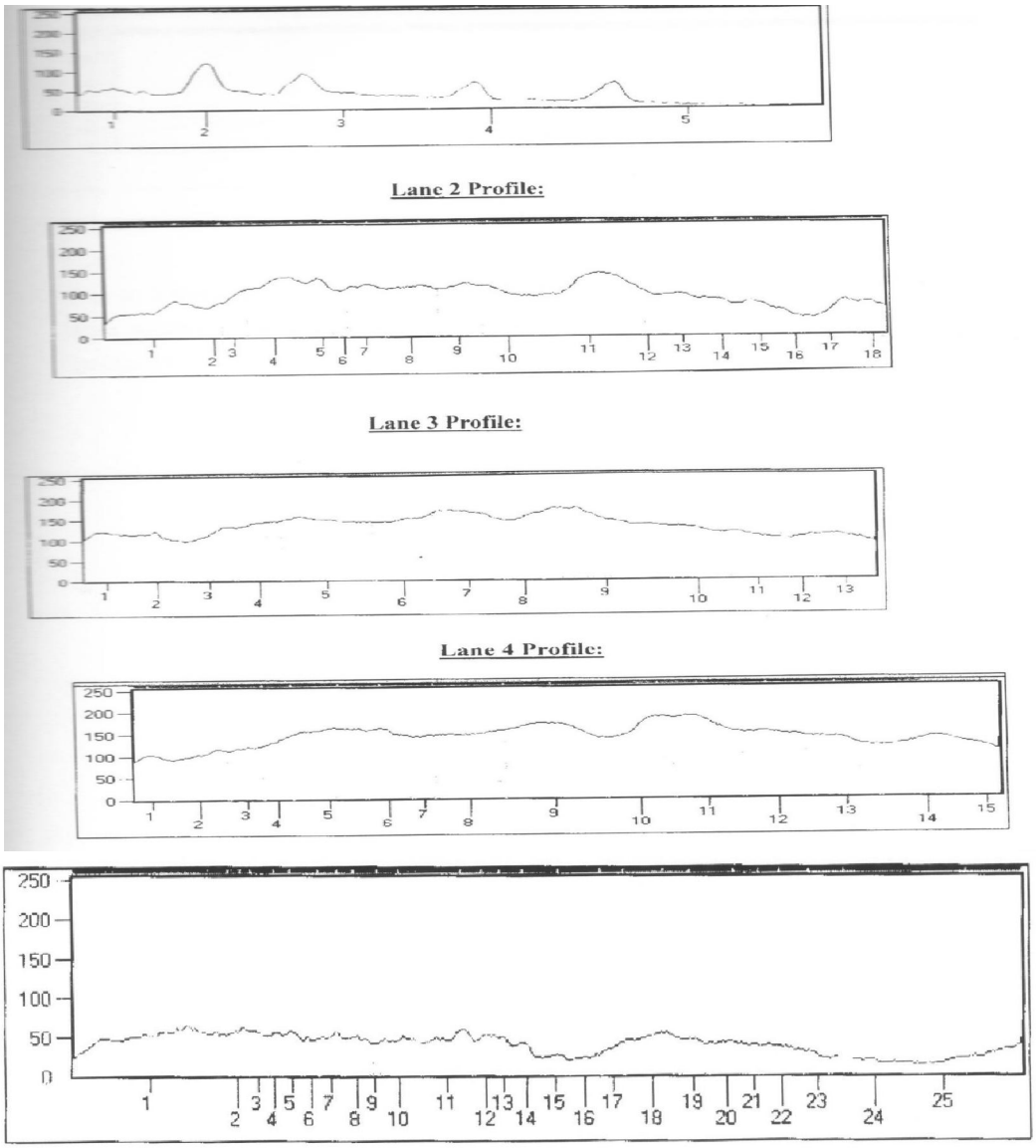


Fig (3): Densitometric analysis of protein using software data analysis for gel proanlyzer 3.0 and IBM compatible personal computer.

4. Discussion

Multidrug resistance (MDR) is emerging problem in the clinical management of bacterial infections **(Balkhair et al., 2014).** The ongoing explosion of antibiotic-resistant infections continues to plague global and US health care. Meanwhile, an equally alarming decline has occurred in the research and development of new antibiotics to deal with the threat **(Spellberg et al., 2008)**. Enterobacteriaceae isolates resistant to multiple antibiotics have been reported from several parts in the world **(Hall et al., 2003)**

Plasmid DNA profile can be used as an epidemiological marker, but in combination with other typing methods and with antibiotic resistance patterns to characterize the circulation of clinical strains and the spread of resistance in these bacteria **(Millesimo et al, 1996; Miller and Diep, 2008).**

The results of this study revealed that among four chosen multidrug resistance strains *S. aureus, E. coli, K. pneumoniae and P. aeruginosa* plasmid was detected with sized from 83 - 175 Kbp. This was in accordance with **Gerhardt (1994).**

demonstrated that MDR E. coli and K. pneumoniae isolates harbored significantly more (≥3) plasmids compared to their non-MDR counterparts, which carried ≤2 plasmids ($p<0.01$) **(Huang et al., 2012).**

The plasmids isolated from MRSA strains were mostly smaller than 7601 bp, the other measured plasmids were M.W 7 601 bp and thus may be considered responsible for chloramphenicol, tetracycline and erythromycin resistance **(Novic, 1989)**.

Our results in agreement with that found by **Skurray et al., (1988)** who found that, Australian MRSA isolates may harbor up to three different plasmids, small (1.6-Kb) cryptic plasmids, 4.5-Kb chloramphenicol resistance plasmids, and 20-42 Kb plasmids which encoded resistance to antiseptics, disinfectant, trimethoprim, penicillin, gentamicin, tobramycin, and kanamycin. Our study found that the tested MRSA strains contained plasmids, but the strains did not detect common plasmids that correlated with multidrug resistance in any isolated Gram-positive cocci species.

Our results were not in accordance with **Mowafy et al., (1990), and Grubb (1990)t**hey concluded that, there was a lack of correlation between Plasmid profiles and multidrug resistance could be explained that many of the resistance determinants were chromosomally mediated. On the other hand, some of R determinants were part of the transposable DNA sequences that can integrated into the undergo translocation to multiple chromosomal and plasmid sites **(Fluit et al., 2001; Tohamy et al., 2006; Malachowa & Deleo,2010; Karthikeyan et al., 2010).**

Also plasmids can be spontaneously lost from readily acquired strain as they are extra chromosomal and are not part of the chromosomal genotype that defines the host strains **(Arebeit, 1999)**.

The protein finger print technique was carried out for the selected isolates to confirm the identification of isolates. Our results showed that, in the electrophoregram (scan of protein profiles or finger prints of the total cellular proteins of the vegetative cells of tested isolates (No.19 u, 20 u, 10 u and 30 s) were fractionated in denatured gel by electrophoresis (SDS-PAGE) and the protein bands revealed from 13 to 25 discrete protein bands. The protein profiles isolates (No 19 u. 20 u, 10 u and 30 s) contains 18, 13, 15 and 25, respectively, showed that, the comparison of protein patterns from 4 isolates were similar especially in the high molecular weight parts of the gels except the last isolate no (30 s) which diverse from the other isolates which the highest molecular weight was 126.029. Most of the bands in each isolate could be matched with bands in each of the others.

Diab et al., (2004) found that, certain protein bands were observed in *S. aureus*. Two bands with molecular sizes of 105 and 55 Kbp were detected before and disappeared after plasmid curing. The disappearance of such protein bands with the plasmid curing and loss of antibiotic resistance comprises a definite observation and good evidence to suggest a possible co-relation. The disappearance of some bands in the protein pattern of plasmid-cured isolates indicates these protein genes are either located in plasmid DNA like ß -lactamases genes or as chromosomal genes but under the control of one or more plasmid genes.

References

Aiello, AE, Lowy FD, Wright, LN, Larson, EL (2006). Meticillin- resistant *Staphylococcus aureus* among US prisoners and military personnel: review and recommendations for future studies. Lancet Infect. Dis. 6:335-341.

Alarn. K, Maven. H., Taie R, Rao. K. S, Burrows.I., Huber M E, Raki.T (1994). Preliminary screening of seaweeds, sea grass and lemongrass oils from papua New Guinea for antimicrobial and antifungal activities.International Journal of microbiology..32 (4):396 -399.

Al-Bayati FA (2008). Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts. J. Ethnopharmacol.March..116(28): 403-40**.**

Arbeit, RD (1999). Laboratory procedure for epidemiologic analysis of microorganisms. P.116-

131. In Murray, P. R., Baron, EW.J.; Pfaller, M. A.; Tenover, F. C.; Yolken, R. H.(ed), Manual of Clinic. Mocrobio. 7th ed. ASM press Washington, D.C.

Balkhair A., Al-Farsi Y.,Al-Muharrmi Z., Al-Rashdi R., Al-Jabri M., Neilson F., Al-Adawi S., El-Beeli M., and Al-Adawi S., (2014). Epidemiology of Multi-Drug Resistant Organisms in a Teaching Hospital in Oman. The Scientific World J. Article ID 157102, 6.

Bonfiglio G, Carciotto V, Rnsso, G, Stefani S (1998). Antibiotic resistance in P. aeruginosa, An Italian Survey. J. Antimicrob. Chemother. 5(7): 307-310.

Cirioni, O, GMselli, R., Silvestri, C, Kamysz, W, Orlando, F, Giacometti.Mocchegiani F, DiMatteo F, Riva A, Lukasiak, J, Scalise, G, Saba V (2007). Tachyplesin 111, Colistin, and Imipenem against a multiresistant Pseudomonas Efficancy aeruginosa Strain.. Antimicrob Agents Chemother. 51: 2005- 2010.

Collee J G, Duguid J P, Marmin BP (1989). Mackie and MacCarthey. Practical Medical Microbiology 13th ed. Churchill Livingstone, Edinburgh, London. 2: 141-160.

de Lencastre, H, Oliveira D, Tomasz A (2007). Antibiotic resistant: a paradigm of adaptive power. Curr. Opin. Microbiol.. 10:428-435.

Diab, A M, Selim S A, El alfy S M, Abdelrahmmaii, A A (2004). Plasmid and outer membrane proteins (OMPS) correlation with antibiotic resistance in bacterial eye infection. N. Egypt. J. Microbial. 9: 152-161.

(Domenico P, Marx J L, Schoch P E, Cunha B A (1992). Rapid plasmid DNA isolation from mucoid Gram-negative bacteria. J.Clin. Micro. 88(2): 308-316.

El Behedy EM, Shaheen AA, Amer FA (2000). Comparison of phenotypic and genotypian methods for detection of methicillin- resistant Staphylococcus aureus. countries in south and east M Asia.M.D. Thesis, Microbiology and Immunology Department Faculty of Medicine Zagazig University, Egypt.

Fluit D C, Visser M R., Schmitz S R (2001). Molecular detection antimicrobial resistance. Clin. Microb. Rev. 14(4):836-871.

Gerhardt P (1994). Methods for general molecular bacteriology. Amer. Soc. Microb. 15(3):44-50.

Hall LM, Blok M, Paauw A, Fluit A C, Verhoef F (2003). Multidrug resistance among Enterobacteriaceae is Strongly Associated with the presence of integrons and is independent of species or isolate origin. J. Infect Dis. 187(2): 251-259.

Hamouda H M (2000). Studies on antibiotic degradation by antibiotic-resistant bacteria. M. Sc. Thesis, Department of Botany, Faculty of science, Zagazig University, Egypt.

Hartinger J., Stenius K., Högemann D. and Jahn R. (1996).16-BAC/SDS–PAGE: A Two-Dimensional Gel Electrophoresis System Suitable for the Separation of Integral Membrane Proteins. Analytical Biochemistry. 240(1).126-133.

Haung V, Ryback J M (2005). Pharmacodynamic of cefepime alone and in combination with various antimicrobial s against methicillin resistant S. aureus in an itro pharmacodynamic infection model. Antimicrobial agents and Chemotherapy. 49(1): 302 -308.

Huang Z, Frye J, Chahine A et al., (2012). Characteristics of Plasmids in Multi-Drug-Resistant Enterobacteriaceae Isolated during Prospective Surveillance of a Newly Opened Hospital in Iraq. journal.pone.0040360

.**Hughes WT, Armstrong D, Bodey, G P (2002).** Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clinical Infectious Disease. 34: 730-51**.**

Jarvis WR, Schlosser J, Chinn R Y, Tweeten, S, Jackson M (2007). National prevalence of methicillin-resistant Staphylococcus aureus in inpatients a US health care facilities, 2006. Am. J. Infect. Control. 35:631-637.

Jeljaszewicz J, Mlynarczyk G, Mlynarczyk A (2000). a Antibiotic resistance in Gram positive cocci. Int. J. Antimicrob. Agents.16: 473-478.

Jones RN (2003). Global epidemiology of antimicrobial resistance among community acquired and nosocomial pathogens: A five year summary from the SENTRY antimicrobial surveillance program (1997- 2001). Semin. Respir. Cril. Care Med. 24(1): 121- 134.

Karthikeyan K K, Mark A T, Timothy R T, Jay B, Fafhana B T, Ravikumar B, Uma C, Michel D, et al (2010). Emergence of a new Antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study**.** The Lancet Infectious Diseases. 10(9):597-602.

Klein E, Smith D L, Laxminarayan R (2007). Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999- 2005. Emerg. Infect. Dis. 13:1840-1846.

Klevens R M (2007). Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA. 298:1763-1771.

Malachowa N, Deleo F (2010): Mobile genetic elements of Staphylococcus aureus.Cell Mol Life Sci. 67(18): 3057-3071.

Maniatis T, Fritsch EF, Sambrook. J (1982). Molecular cloning a Laboratory Manual 3rd. ed. Cold Spring Harbor, Ny. suppl. 1:78- 85.

Miller L G, Diep, BA (2008). Clinical practice: colonization, fomites, and virulence: rethinking

the pathogenesis of community-associated methicillin- resistant *Staphylococcus aureus* infection. Clin. Infect. Dis. 46:752-760.

Millesimo M., Intinis, G.D, Chirillo. M G, Mussot T., Savoia, D (1996), Pseudomonas aeruginosa clinical isolates: serotypes. resistance phenorypes and plasmid profiles. Eur J. Epidermol.12: 123-129.

Mowafy H, El-Sharkawy A., El-Baz N, Shheen A, Gawish A (1990). Antimicrobial resistance and plasmid content in clinical and environmental Pseudomonas aeruginosa. Az.J. Micro.. 9(2): 79-92.

Nickerson E K, Eoin West T, Day N P, Peacock SJ (2009). Staphylococcus aureus disease and drug resistance in resource-limited The Lancet Infectious Diseases. 9 (2): 130 – 135.

Nickerson E K, Eoin West T, Day N P, Peacock Waldron D., Lindsay J.,(2006). Microarrays reveal that each of the ten dominant Lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. J. Bacteriol. 188:669-676.

Novic R.P (1989). Staphylococcus plasmids and their replication. Annu. Rev. Microbio. 43: 537-565**.**

Roychouldhury S, Catrenich C E, Mcintosh E H., Mckeever H. D, Makin K. M Koenigs P M (2001). Quinolone resistance in Staphylococcus: activities of new nonflourinated against molecular targets in whole cells and clinical isolates. Antimicrob. Agent Che. 45(4): 1115-1120.

Sambrook J, Frtrsch E F, Maniatis T (1989). Molecular Cloning, A laboratory Mannual, 2nd ed. Cold spring Harbor. 96: 543-567.

Skurray P A, Rouch DA, Lyon B.R., Gillespie M.T, Tennent J.M, Byrne M.E, May J W (1988). Multiresistant staphylococcus aureus genetics and evaluation of epidemic Australian strain.J. Antimicrob. Chemo..21: 19-38.

Tohamy E V, Abo-Zeid A M, Shaheen A A, El-Awadi S F (2006). Nosocomial Infection in Surgical Hospital in Zagazig University. Arab Univ. Cairo. 14(1). 133-145..

Tompkins, TL S (1985). DNA methods in clinical microbiology in: E.H. Lennet, A., Balows, W.J. Hauster, Jr. and Shadomy H.I.(ed), Manual of clinical microbiology, 41th Ed. American Socity for Microbiology,Washington, D.C., pp. 1023-1028.

Udo E E, Grubb WB (1990). Excision of a conjugative plasmid from The Staphylococcus chromosome. J. Med. Microb. 31:207-212.

Waldron D., Lindsay J.,(2006). Microarrays reveal that each of the ten dominant Lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. J. Bacteriol. 188:669-676.

Waterer G W, Wunderink R G (2001). Increasing threat og Gram – negative bacteria.Crit. Care. Med. Apr. 29(4): 75-81.

9/12/2014