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Protein and DNA fingerprints of Some Multidrug-Resistant Bacteria

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Abstract: Multidrug resistance is a major threat to the fighting of infectious bacterial pathogens. This is because they turn the current generations of antibiotics into obsolete non-effective compounds. Therefore the search for new antibacterial compounds is complicated because of the price tag and the time needed for marketing. Nine pathogenic bacteria were tested for their resistance to 19 different antibiotics and studied at molecular levels using protein banding patterns, DNA fingerprinting, when grown at optimum and elevated temperatures. The eight strains belonging to five genera of bacteria were resistance to the 19 drugs was evident with Aeromonas sp., E. coli, Klebsiella pneumonia, Proteus mirabilis, and Pseudomonas aeurginosa. Only Klebsiella pneumonia strain 1 showed sensitivity to the meropenem disc saturated with 10ug of the drug. The protein banding patterns discovered the minute differences between the two isolates of Psuedomonas, Klebsiella and E. coli. Moreover, the DNA fingerprints using RAPD-PCR distinguished between the two Klebsiellia strains and E. coli strains. In conclusions the use of molecular biology tools can be helpful in distinguishing between closely related strains of the same bacterial pathogen species.

keywords: Multidrug, resistance, bacteria, antibiotic, fingerprinting

1.Introduction

The multidrug resistance to antibiotics of the ever-increasing number of bacterial pathogens threatens the effective treatment of these microbes by the existing antibiotics. This is a huge public health threat at the global level which requires not only attention but also immediate actions. This compromises the health care industry and put tremendous burden and concerns on the societies since everybody is prone to illness and microbial infection. As reported by the World Health Organization, multidrug resistance already starts to complicate the fight against tuberculosis (TB), human immunodeficiency virus (HIV), and malaria, as well. Not only the lack of effective antibiotics will compromises the health and success of major surgeries, organ transplantation, and cancer chemotherapies, but also cesarean sections or hip replacements) become very high risk [5, 8, 11, 22]

Resistance to the most advanced generations of antibiotics such as carbapenem, fluoroquinolone, third generation cephalosporin, colistin, extra drug resistant tuberculosis (XDR-TB), has been reported all over the world. This multidrug resistance was most evident in the life-threatening infections by many bacterial pathogenic agents such as *Klebsiella pneumoniae*, *E, coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Neisseria gonorrhoeae*. ..etc. There are variety of reasons which contribute to the infection by these dangerous pathogenic bacteria; these microbes can be acquired in hospital settings (nosocomial), due to life-style (the sexually transmitted) or even casual contacts.

A famous example of the widespread resistance to antibiotics and hence the treatment failure against gonorrhea was reported by the WHO in 10 developed countries. Some of these countries are located in the heart of Europe (Austria, France, Norway, Slovenia, Sweden, and the United Kingdom) and others are outside Europe (Australia, Canada, Japan, and South Africa). A 64% more death among patients infected with the methicillin-resistant *S. aureus* (MRSA) is a catastrophic illustration

of MDR problems. Moreover, a recent generation of antibacterial agents called colistin became useless in the fight against bacterial pathogens belong to the family Enterobacteriaceae, which cause many nosocomial infections. Since 1980 this drug has been used effectively to fight various Gram-negative rod-shaped Gram-negative MDR bacteria such as *Acinetobacter baumannii, K. pneumonia*, and *P. aeruginosa* [6]

Bacteria resistance to numerous drugs was proved to be due to gaining plasmids and or transposons carrying genes encode for resistance to certain antibiotic or drug. The type of resistance could be varied and starts with resistance to one drug and starts to buildup to reach resistance to several drugs; i.e. accumulated resistance which is now termed multidrug resistance. These acquired genes could be inactivating the drug or preventing the uptake of the drug or pumping out the up taken ones. The current knowledge and concerns are huge because of the implication and serious consequences associated with this phenomenon [6 16, 18, 25].

Without the proper understanding of the reasons leading to the emergence of the MDR, the consequences will be prolonged illness, disability, and death. Therefore the objective of this study was to generate enough information about the nature of the MDR microbes to aid in understanding and better treatment protocols of these dangerous bacterial pathogens.

2. Materials and methods

Bacteria and growth media

Eight multidrug resistant bacterial human pathogens (MDR) were obtained from the Microbiology Department, Faculty of Medicine, Mansoura University. These were two isolates of each of *E. coli, Klebsiella pneumonia, Proteus mirabilis,* and *Pseudomonas aeurginosa;* and one *Aeromonas* sp. These microbes were grown in different media such as Luria Bertani (LB) contains g/L: Tyrptone 10g, yeast extract 5g, Sodium chloride 10g and agar 15g), Nutrient Agar (Peptone 5g, yeast extract 3g, sodium chloride 5g, and agar 15g), Cetrimide Agar (gelatin 20g, $MgCl_2$ 1.4g, Potassium chloride 10g, cetyltrimethylammonium bromide 0.3g, glycerol 10ml, and 15 g agar. pH 7.2), McConkey agar (peptone 17g, proteose peptone 3g, lactose 10g,bile salt 1.5g, sodium chloride 5g, neutral red 0.03g, crystal violet 0.001g, and agar 15g), Mueller Hinton Agar (2.0g beef extract, 17.5g casein hydrolysate, 1.5g starch, and 17.0g agar) and Blood agar (Pancreatic digested casein 15.0 g, Papaic digest of soy meal 5.0 g, NaCl 5.0 g, and Agar 15.0 g). All bacterial isolated grown in solid/liquid media were incubated at 37^0C for overnight before any further manipulations.

Antibiotic sensitivity test

All nine MDR bacterial species were tested for their sensitivity to 19 commercially available antibiotic discs by Kirby-Bauer disc diffusion method [2]. Each disc type was saturated with a fixed concentration of the antibiotic and used as recommended by the manufacturer (Table 1). The zones of inhibitions were recorded and analyzed according to the published data of the Clinical Laboratory Standard Institute (CLSI). The antibiotic discs used and their concentrations were Ampicillin (10ug), Amoxicillin (25ug), Amoxycillin- Clavulanic acid (20-10ug), Aztreonam (30ug), Cefoxitin (30ug), Cefsoludin 30, Cephalexin (30ug), Chlorampheicl 30, Erythromycine (15ug), Imipenem (10ug), Kanamycin (30ug), Meropenem (10ug), Methicillin (10ug), Nalidixic Acid (30ug), Oxacillin (5ug), Penicillin (10ug), Streptomycin (10ug), Trimethoprim- sulfamethoxazol (1.25-23.75ug), and vancomycin (30ug) [2, and 3]

Curing of MDR

The MDR bacterial pathogens included in the study were grown at three different temperatures; 37, 40 and 45C [13, 19]. These strains were tested for their sensitivity to different antibiotics by the disc diffusion method [2,3].

Protein banding patterns

The protein fingerprints of all bacterial isolates were obtained by fractionation in denatured polyacrylamide gels as described by Laemmli (12). The denatured polyacrylamide gels contain sodium dedocylsulphate (SDS) and consisted of a resolving or separating gel and stacking. All reagents of both layers were combined de-aerated without the polymerization catalysts:

tetramethylethylenediamine and ammonium persulphate (TEMED and APS), which were added immediately prior to casting the gels. Bovine serum albumin (BSA) was used to construct a standard curve for protein quantification.

DNA Fingerprinting

DNA fingerprinting was done according to standard published protocols [1, and 21] with adjustments of the annealing temperature depending on the G+C content of each primer used. The random primers used were OP-U16, and OP-T16 obtained from Operon Company, USA. The PCR volume of 25ul contained 20 ng of bacterial template DNA, 20 pmol of each primer, 1U of Taq DNA polymerase (enzynomics, Korea), and 250uM (each) dCTP, dGTP, dATP, and dTTP (enzynomics, Korea) in 10 mM Tris-Cl (pH 8.3)-50 mM KCl-0.1% Triton X-100 under a drop of mineral oil. The cycling program used for amplification was 35 cycles started with a single denaturation step at 94°C for 5 min. Each of the 35 cycles consisted of heating to convert dsDNA into ssDNA at 94°C for 1 min, 46°C for 1 min and ended with extension at 72°C for 3min. A final extension step was done at 72°C for 7 min. The PCR products were separated and visualized agarose gel electrophoresis and then documented by photography

3. Results and Discussion

Antibiotic sensitivity

The *Aeromonas* sp. showed multidrug resistance to all antibiotics tested. No inhibition zones produced by the discs of ampicillin, amoxicillin, azetronam , calavalanicacid , cephalexin, cefsoludin , cefoxilin, chlorampheicl, , kanamycin ,methicillin oxacillin , penicillin , streptomycin, trimihoprem-sulfamethyazol , imipenem, and vancomycin. The amoxicillin and meropenem showed bacteriostatic effects on *Aeromonas* or pseudo-sensitivity because bacteria regrow within the inhibition zone after 24 hours. While the inhibition zones appearing around the central disc in Fig. (1) are less than 16 mm in diameter and according to CSLI are considered resistant. The ***E. coli*** was sensitive to four antibiotics chloramphenicol, meropenem, nalidixic acid and trimethoprim-sulfamethoxazol. It showed intermediate sensitivity to imipneom, cefsoludin (CEs) with inhibition zones ranged from 16 to 21mm in diameters and resistance to ampicillin, amoxicillin, aztreonam, clavulanic acid, cefoxitin, cephalexin, erythromycin, kanamycin, methicillin, oxacillin, penicillin, streptomycin, and vancomycin. The *K. pneumonia* strain one (K3) showed variable responses to the antibiotics tested. This strain of bacteria was sensitive to meropenem, intermediate sensitivity to cefoxillin and resistance to the other 17 antibiotics tested; multidrug resistance. However, *K. pneumonia* strain two (K4) grown at 37°C showed resistance to all tested antibiotics except cefoxillin and clavulanic acid. It showed intermediate sensitivity to clavulanic cacid high sensitive to cefoxillin. The two strains of *Proteus mirabilis* (1 and 2) showed multidrug resistant to all tested antibiotic discs when grown at 37°C. Finally, each of the two strains of *Pseudomonas aeurginosa* strains one and two showed resistance to 18 of the 19 antibiotics used in this study. *P. aeurginosa* strain one was sensitivity to cefoxillin and *P. aeurginosa* strain two showed sensitivity to meropenem (Fig. 1 and Table (1))

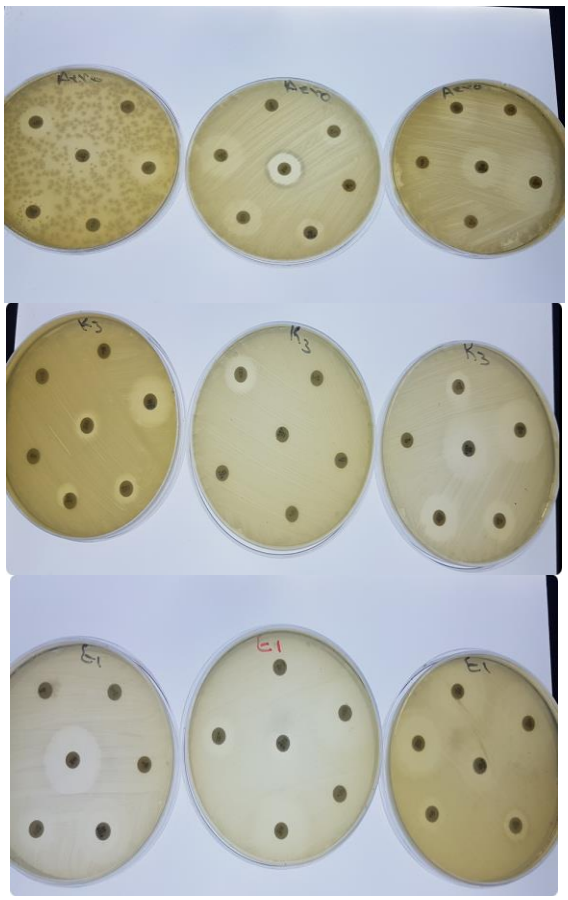

Fig. 1. The antibiotic sensitivity of *Aeromonas (Aero) E. coli* (E1), *K. pneumonia*e 3 (or strain 1) and *P. aeruginosa* strain 1 grown at 37°C as determined by the disc diffusion method

Table (1) The antibiogram of all eight bacterial pathogens studied as recommended by the CLSI.(2012)

Antibiotics	**E1**	**Ps1**	**Ps2**	**Pr1**	**Pr 2**	**Ar**	**K3**	**K4**
Ampicillin (10ug)	R	R	R	R	R	R	R	R
Amoxicillin (25ug)	R	R	R	R	R	R	R	R
Amoxycillin- Clavulanic acid (20-10)	R	R	R	R	R	R	R	I
Aztreonam (30ug)	R	R	R	R	R	R	R	R
Cefoxitin (30ug)	R	R	R	R	R	R	R	S
Cefsoludin (30ug)	I	R	R	R	R	R	R	R
Cephalexin (30ug)	R	R	R	R	R	R	R	R
Chloramphenicol (30ug)	S	R	R	R	R	R	R	R
Erythromycine (15ug)	R	R	R	R	R	R	R	R
Imipenem (10ug)	I	R	R	R	R	R	R	R
Kanamycin (30ug)	R	R	R	R	R	R	R	R
Meropenem (10ug)	S	S	R	R	R	R	S	R
Methicillin (10ug)	R	R	R	R	R	R	R	R
Nalidixic Acid (30ug)	R	R	R	R	R	R	R	R
Oxacillin (5ug)	R	R	R	R	R	R	R	R
Penicillin (10ug)	R	R	R	R	R	R	R	R
Streptomycin (10ug)	R	R	R	R	R	R	R	R
Trimethoprim- sulfamethoxazol(1.25-23.75ug)	S	R	R	R	R	R	R	R
Vancomycin(30ug)	R	R	R	R	R	R	R	R

Genetic stability

Normally, the MDR bacteria used in this study grew well at 37°C as the human body temperature. Retaining resistance to the antibiotics after growth at higher temperatures other than the optimum (37°C) such as 40°C and 45°C was taken as a measure of genetic stability. This curing experiment showed the responses of the seven MDR bacterial pathogens to the antibiotic used during the study. It is noticeable that the sensitivity patterns of the examined MDR bacterial pathogens had changed at elevated temperature. At 40°C *Aeromonas* species were sensitive to Trimithoprim-Sulfamethoxazol, chloramphenicol, ampicillin. *E. coli* strain one (E1) was sensitive to aztreonam, kanamycin, cephalexin, chloramphenicol, Trimithoprim-Sulfamethoxazol, *E. coli* strain two (E2) was sensitive to cefoxitin, aztreonam, nalidixic, erythromycin. *K. pneumonia* strain one (K3) was sensitive to amoxicillin, kanamycin, ampicillin, imipenem, erythromycin, nalidixic, oxacillin, cephalexin, chloramphenicol, methicillin. *P. mirabilis* strain one (Pro1) was sensitive to kanamycin, nalidixic acid, Trimithoprim-Sulfamethoxazol P. mirabilis strain two (Pro2) was sensitive to methicillin, nalidixic, kanamycin, penicillin, ampicillin, imipenem, Trimithoprim-Sulfamethoxazol, cephalexin amoxicillin. *P. aeruginosa* (Ps1) became sensitive to aztreonam, cephalexin, kanamycin, imipenem, *P. aeruginosa* (Ps2) was sensitive to kanamycin, cephalexin, imipenem. The same patterns were seen at 45°C.

Protein fingerprints of MDR strains

A deep analyzing look at the protein banding patterns of the nine bacterial pathogens showed the distinctive differences among all of them; despite the apparent similarities in these protein patterns. Moreover, every two isolates of the same bacterium like *P. aeuroginosa, K. pneumonia, E. coli, and Proteus mirabilis* produced the same proteins with the same electrophoretic motilities and showed differences in one or two bands at most as indicated by the arrows in Fig. (2); *P. aeuroginosa* strain 2 lacks a band close to 45kDa compared to strain 1. The two strains of *K. pneumonia* 1 and 2 differ in two bands as indicated by the arrows, they are present in K1 and absent from the banding pattern of K2. *E. coli* 2 lacks one band, and each of the two strains of *P. mirabilis* lacks one band than the other strain

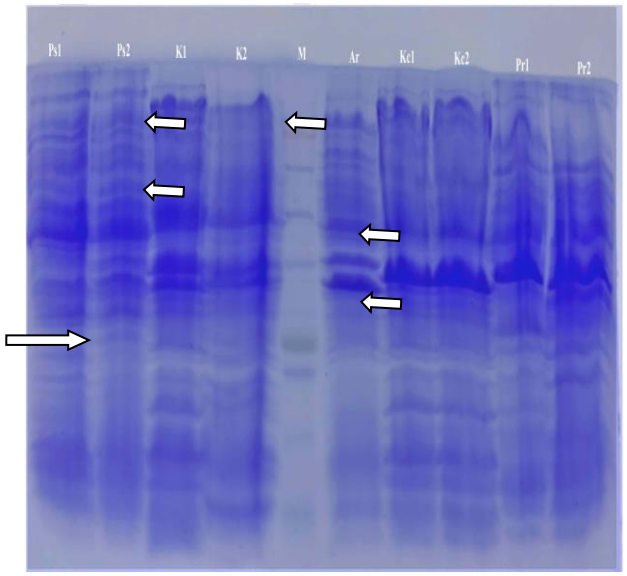

Fig. 2. Protein banding patterns of nine MDR bacterial pathogens grown at 37°C. Lane Ps1 and Ps2: *P. aeruginosa* 1 and 2 isolates; lanes K1-K2*: K. pneumonia* 1 and 2 strains; lane M: protein molecular weight marker (245, 180, 135, 100, 75, 63, 48, 35, 25, 20, 17, and 11kDa); lane Ar: *Aeromonas* sp., lane Kc 1-2: *E. coli* strains 1 and 2 and lane Pr1 and Pr2: *Proteus mirabilis* 1 and 2 strains.

DNA Fingerprinting of MDR strains

The only differentiating power was realized by using some random oligonucleotide primers from the Operon kits. One such primer was able to distinguish between the two closely strains of *Klebsiella pneumonia* and *E coli* as seen in (Fig. 3). However, no distinctive DNA fragments were produced by this primer with *Aeromonas*, and the two strains of each of the *Pseudomonas* isolates, and *Proteus* isolates 1 and 2

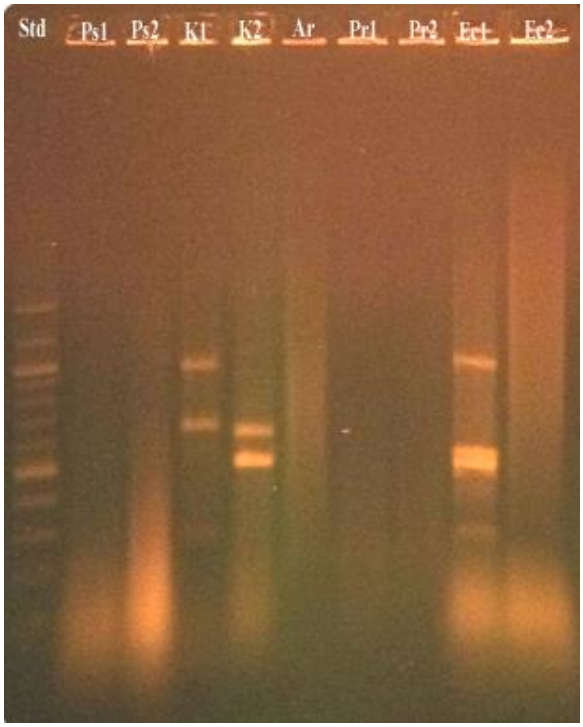

Fig. 3. DNA fingerprint of the nine multidrug resistant bacterial pathogens. Panel Std: DNA molecular weight size marker (1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100bp), lanes Ps1, Ps2, K1, K2, Ar, Pr1, Pr2, Ec1, and Ec2: *P. aeruginosa* 1, 2, *Klebsiellia pneumonia* 1 and 2, *Aeromonas*, sp., *Proteus mirabilis* 1 and 2, and *E. coli* 1 and 2, respectively

Discussion

Molecular techniques are more candid to get deep insights into living cells such as bacteria. Two of the major techniques used with the nine MDR bacterial pathogens throughout this study were protein banding patterns and RAPD-PCR. Both techniques were able to indicate the minute differences between the strains of the same species. The two strains of *P. aeurginosa* differed by the presence of a single band in strain 1 not in strain 2 and so the cases between the two strains of *K. pneumonia* and *E. coli*. Moreover, the DNA fingerprints of the bacterial pathogens included in this study showed that RAPD-PCR technology is a differentiating power that is unique to each isolate even with the same primer. The number of DNA fragments produced with each primer and the intensities of these fragments is enough to

distinguish between two strains of the same bacterial pathogen such as *Klebsiella pneumonia* and *E. coli*. The primer used was able to show a common distinctive DNA band characteristic to the species and extra bands to indicate two different strains. As been widely documented in the literature the positional effect to which the primer annealed determines the size of the fragment of DNA amplified. This technique showed the diversity between two isolates belongs to the same species of either *E. coli* or *Klebsiella pneumonia.* Our data about RAPD-PCR was supported by Bassam, et al in 1992 who concluded that "amplification produces a characteristic spectrum of products that is adequately resolved by gel electrophoresis and visualized by staining". The discriminatory character of the RAPD-PCR was highlighted during the typing of not only isogenic morphotypes of *V. vulnificus* but during the typing of many other bacteria such as *Campylobacter coli, C. jejuni, Listeria monocytogenes,* and *Staphylococcus haemolyticus* [15, 21, and 20].

Another prominent case was that of health problems associated with the MDR *Acinetobacter baumannii* causes the serious nosocomial infection ventilator-associated pneumonia (VAP) that is hard to treat and control. Moreover, the infected patients became a reservoir for the spreading fast of this pathogenic agent to other high-risk people in many south Asian countries. The lack of antibiotic susceptibility had increased the mortality rates among patients because of the failure of treating this disease [5, 9]. Jansen, et al, [10] had studied the MDR resistance of *Pseudomonas* clinical isolates of cystic fibrosis patients. They found that bacterium had developed phenotypic characters and collateral sensitivity. Theoretically, these changes were attributed to pleiotropic effects of the pool of genes causing the resistance. They further reported that 90% of the *Pseudomonas* clinical isolates showed intermediate resistance to one antibiotic at least, of which 15% were multi-drug resistant. Further the suggestion that genetic variants among MDR bacterial pathogens require analysis of whole-genome sequences to determine the interrelationships among isolates accurately [22, 17].

The increasing resistance of *E. coli* and *K. pneumoniae* to the third generation of cephalosporins had forced the physicians to use the most expensive carbapenems to treat these dangerous bacterial pathogens, which may speed up resistance to this last resort drug. Now we have to face the reality that bacterial infections from minor injuries can kill far more infected patients than can be imagined. The WHO has published an alert to the governments around the world about the seriousness of the MDR problem and asked for immediate actions. Therefore, determining the exact identity of the bacterial pathogen and the scope of its resistance to multiple drugs is essential for formulating an effective response by all health officers and governments around the world (8, and 24).

Bacterial resistance to numerous drugs was proved to be due to gaining plasmids and or transposons carrying genes encode for resistance to certain antibiotic or drug. The type of resistance could be varied and starts with resistance to one drug and starts to buildup to reach resistance to several drugs; i.e. accumulated resistance which is now termed multidrug resistance. These acquired genes could be inactivating the drug or preventing the uptake of the drug or pumping out the up taken ones. The current knowledge and concerns are huge because of the implication and serious consequences associated with this phenomenon [6, 10, 11, 16, 18, 25].

Overall we can conclude that the use of non-specific primers with no designed complementarity to any particular sequence will enable them to explore the entire sequence of the template DNA to find a best-fit match. However, the application of RAPD does not require any prior knowledge about specific sequences in the template DNA can be a huge advantage. Because this technique will help identify a single nucleotide DNA polymorphism that might prevent the annealing of the primer and help detect deletions, insertions or a simple repeat locus.

4.References

1. Bassam, B.J., Caetano-Anollés, G. , Gresshoff, P. M. (1992). DNA amplification fingerprinting of bacteria. Appl Microbiol Biotechnol., **38(1)**:70-76.

2. Bauer, A.W., et al. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. *J. Clin. Pathol*.; **45**:493-496.
3. Bauer, M.F., Sirrenberg C, Neupert W, Brunner M. (1996). Role of Tim23 as voltage sensor and pre sequence receptor in protein import into mitochondria. Cell **87(1)**:33-41
4. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS). (2012). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Tenth Edition. M02-A11. CLSI, Wayne, PA.
5. de Kraker, M. E, Jarlier V, Monen, J. C, Heuer, O. E, van de Sande, N, Grundmann, H. (2012). The changing epidemiology of bacteremias in Europe: trends from the European Antimicrobial Resistance Surveillance System. Clin Microbiol Infect. **19(9)**:860-868.
6. de Lencastre H, Oliveira D, Tomasz A. (2007.) Antibiotic resistant Staphylococcus aureus: a paradigm of adaptive power. Curr. Opin. Microbiol. **10**:428–35.
7. Hawkey, P. M., R. E. Warren, D. M. Livermore, C. A. M. McNulty, D. A. Enoch, J. A. Otter, A. P. R. Wilson. (2018). Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party. *J. Antimicrobial Chemother*, **73(3)**:iii2-iii78,
8. Heinz, E.; H. Ejaz, J. B. Scott5, NancyWang , S. Gujaran, D. Pickard, J. Wilksch, H. Cao, I. ul Haq, G. Dougan, and R. A. Strugnell. (2019). Resistance mechanisms and population structure of highly drug resistant Klebsiella in Pakistan during the introduction of the carbapenemase NDM-1. Sci. Reports, 9:2392.
9. Inchai, J., C. Liwsrisakun, T. Theerakittikul, R. Chaiwarith, W. Khositsakulchai, C. Pothirat, (2015). Risk factors of multidrug-resistant, extensively drug-resistant and pandrug-resistant Acinetobacter baumannii ventilator-associated pneumonia in a Medical Intensive Care Unit of University Hospital in Thailand. *J Infect Chemother* 21 570e574.
10. Jansen, G., N. Mahrt, L. Tueffers, C. Barbosa, M. Harjes, G. Adolph, A. Friedrichs, A. Krenz-Weinreich, P. Rosenstiel and H. Schulenburg. (2016). Association between clinical antibiotic resistance and susceptibility of Pseudomonas in the cystic fibrosis lung. Evolution, Medicine, and Public Health. pp. 182–194
11. Kumar S, He G, Kakarla P, Shrestha U, Ranjana KC, Ranaweera I, Willmon TM, Barr SR, Hernandez AJ, Varela MF. **16(1)**:28-43.
12. Laemmli UK (1970). "Cleavage of structural proteins during the assembly of the head of bacteriophageT4". *Nature*. **227 (5259):** 680–685.
13. Letchumanan,V.; Chan, K.; and Lee, L. (2015). An insight of traditional plasmid curing in Vibrio species. Front. Microbiol.,
14. Makino, S. I., Y. Okada, Y., and Maru, T. (1994). PCR-Based Random Amplified Polymorphic DNA Fingerprinting of Yersinia pseudotuberculosis and Its Practical Applications. *JClin. Microbiol*. **32 (1)**: 65-69.
15. Mitchelson, K. R.,and S. Moricca. (2008). DNA fingerprinting methods for microbial pathogens: Application to diagnostics, taxonomy and plant disease management. Integrated Management of Diseases Caused by Fungi, Phytoplasma and Bacteria, 333–364.
16. Nikaido, H. (2009). Multidrug Resistance in Bacteria. Annu Rev Biochem. 2009; **78**: 119–146.
17. Ron, E. Z. (2010). Distribution and evolution of virulence factors in septicemic Escherichia coli. *Int J Med Microbiol*.**300**:367–70.
18. Sun J, Deng Z, Yan A. (2014). Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological

exploitations. Biochem Biophys Res Commun.**453(2**):254-67.
19. Trevors, J. T. (1986). Plasmid curing in bacteria. FEMS Microbiol. Rev, **1(3-4)**:149–157,
20. van Belkum, A. (2003). High-throughput epidemiologic typing in clinical microbiology. Clin Microbiol Infect; **9**: 86–100.
21. Vaneechoutte, M. (1996). DNA fingerprinting techniques for microorganisms. A proposal for classification and nomenclature. Mol Biotechnol. ;**6(2)**:115-42.
22. Wiles T, Kulesus R, Mulvey M. (2008). Origins and virulence mechanisms of uropathogenic Escherichia coli. Exp Mol Pathol. **85**:11–9.
23. Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A.
24. Rafalski, and S. V. Tingey. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Acids Res. **18(22)**:6531-5.
25. World Health Organization. (2002). Susceptibility testing of Salmonella using disk diffusion". Lab. Protocol Level 2 Training Course. 3rd Ed. by: Rene S. Hendriksen (DFVF)
26. Zechini B, Versace I.(2009). Inhibitors of multidrug resistant efflux systems in bacteria. Recent Pat Antiinfect Drug Discov.; **4(1)**:37-50.