Polish Journal of Microbiology
2010, Vol. 59, No 3, 207–212

ORIGINAL PAPER

Antibiotic Susceptibility and Genotype Patterns of *Escherichia coli*, *Klebsiella pneuomoniae* and *Pseudomonas aeruginosa* Isolated from Urinary Tract Infected Patients

M.I. ABOU-DOBARA[1]*, M.A. DEYAB[1], E.M. ELSAWY[2] and H.H. MOHAMED[2]

[1] Faculty of Science (Damietta), Damietta Branch, Mansoura University and
[2] Urology and Nephrology Center, Mansoura University, Egypt

Received 13 October 2010, revised 1 May 2010, accepted 15 May 2010

Abstract

Thirty nine isolates of *Escherichia coli*, twenty two isolates of *Klebsiella pneumoniae* and sixteen isolates of *Pseudomonas aeruginosa* isolated from urinary tract infected patients were analyzed by antimicrobial susceptibility typing and random amplified polymorphic DNA (RAPD)-PCR. Antibiotic susceptibility testing was carried out by microdilution and E Test methods. From the antibiotic susceptibility, ten patterns were recorded (four for *E. coli*, three for *K. pneumoniae* and three for *P. aeruginosa* respectively). Furthermore, genotyping showed seventeen RAPD patterns (seven for *E. coli*, five for *K. pneumoniae* and five for *P. aeruginosa* respectively). In this study, differentiation of strains of *E. coli*, *K. pneumoniae* and *P. aeruginosa* from nosocomial infection was possible with the use of RAPD.

Key words: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas areuginosa*, antibiotic susceptibility, urinary tract infection

Introduction

Urinary tract infections (UTIs) are most prevalent among geriatric and critically ill patients and occur more commonly after urinary catheterization (Turck and Stamm, 1981; Centers for Disease Control, 1983). Many bacterial species are associated with this infection, including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Gales *et al.*, 2000; Gupta *et al.*, 2007). Multi-drug resistance of antimicrobial classes is common among the uropathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Yuksel *et al.*, 2006; Foda, 2008).

Among whole genome fingerprinting PCR methods, random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990) is used for demonstrating differences between bacteria. RAPD can be used for typing of organisms without previous knowledge of DNA sequences. The use of a single primer leads to amplification of several DNA fragments randomly distributed throughout the genome. The primers used in RAPD are short, usually 8–12 mers, with random sequence composition (Lübeck and Hoorfar, 2003). RAPD has received considerable attention in recent years as a molecular typing method due to its simplicity, sensitivity, flexibility and relatively low cost (Welsh and McClelland, 1990; Williams *et al.*, 1990; Belkum, 1994). The ability of RAPD to type a wide variety of bacteria strains in a short time suggests that it will be a useful molecular epidemiological tool (Gori *et al.*, 1996; Hilton and Penn, 1998; Bosi *et al.*, 1999; Patton *et al*., 2001). Typing helps in the identification of environmental sources as well as indicating whether transmission of strains has occurred between patients (Kerr *et al.*, 1995). It also reveals whether strains emerging after antibiotic therapy are variants of the original or newly acquired strains (Chit and Chew, 1993).

Knowledge of the spread of specific strains is of epidemiological importance in order to monitor the broad spreading of both their pathogenicity and multiresistance (Lopes *et al.*, 2005). The aim of this study was to determine antibiotic susceptibility and to apply RAPD typing method for characterization of the isolated bacterial pathogens.

* Corresponding author: M.I. Abou-Dobara, Botany Dept., Faculty of Science, New Damietta, Damietta P.O. Box 34517, Egypt; **e-mail: aboudobara@yahoo.co.uk or aboudobara@mans.edu.eg**

Experimental

Material and Methods

Collection and transport of the samples. A total of 77 clinical samples were collected from urine samples in sterile tightly locked containers from different hospitalized patients in Urology and Nephrology Center, Mansoura University. The patients were not treated with any of the eight antibiotics tested (Table I).

Table I
Antibiotic types retrieved in this study[a]

Antibiotic	MIC[b]			Conc. Range [µg/ml]	
	S	I	R	Broth	E test
IP	≤4	8	≥16	1 – 16	0.002 – 32
AK	≤16	32	≥64	2 – 64	0.016 – 256
PT	≤16	32	≥128	8 – 128	0.016 – 256
XL	≤8	16	≥32	4 – 32	0.016 – 256
NI	≤32	64	≥128	16 – 128	0.032 – 512
CT	≤8	16	≥64	8 – 64	0.016 – 256
NX	≤4	8	≥16	4 – 16	0.016 – 256
TS	≤2	–	≥4	2 – 4	0.002 – 32

[a] Abbreviations: IP, Imipenem; AK, Amikacin; PT, Piperacillin/tazobactom; XL. Amoxicillin/clavulanate; NI, Nitrofurantoin; CT, Cefotaxime; NX, Norfloxacin; TS, Trimethoprim-sulfamethoxazole;

[b] Susceptibility was performed as described by National Committee for Clinical Laboratory Standards [NCCLS, 2006], which was used to categorize strains as susceptible or sensitive (S), intermediate (I) or resistant (R).

Isolation of the bacterial isolates. The collected samples were inoculated into sterile Petri dishes containing ready prepared Cled agar and blood agar media and incubated at 35°C for 18–24 hours. After the incubation period, the plates were examined for growing bacterial colonies. The isolated colonies were subcultured and purified for characterization.

Identification of the different isolates. The isolated bacteria were identified according to Bergey's Manual of Determinative Bacteriology (Brenner, 1986; Ørskov, 1986a and b; Palleroni, 1986) and confirmation of species identification was carried out by using automated Microscan (DADE BEHRING, USA).

Antibiotics sensitivity tests. The antimicrobial susceptibility tests of the isolated bacteria were carried out using the following methods:

1 – Broth Microdilution MIC Method. A sterile plastic tray containing various concentrations of antimicrobial agents (Table I) was inoculated with a standardized number of test bacteria in Mueller Hinton broth. After overnight incubation at 35°C, the minimal inhibitory concentrations (MIC) were determined and interpreted as susceptible, intermediate, or resistant (Table I).

2 – E Test. The system comprises a E test strip with predefined antimicrobic gradients (Table I), to determine the Minimum Inhibitory Concentration (MIC), in µg/ml of individual agent against microorganisms as tested on agar media (Mueller Hinton Agar). The inoculated media were incubated at 35–37°C for 18–24 hours. After the incubation period the MIC values were recorded at the point of intersection between the inhibition ellipse edge and the E test strip (Table I).

Genotyping of the bacterial isolates

Isolation of the bacterial DNA. Bacterial colonies were removed and suspended in 1 ml distilled water, then centrifuged for 10 min at 5000 × g. DNA was extracted using High Pure PCR Template Purification Kit, Germany) as follow: The bacterial pellets were suspended in 200 µl phosphate buffered saline. 15 µl lysozyme was added and incubated for 15 min at 37°C. Subsequently 200 µl binding buffer and 40 µl proteinase K were added, mixed immediately and incubated for 10 min at 72°C, then 100 µl isopropanol was added to precipitate DNA. The filter tubes and the collection tubes were combined and the samples were pipetted, and then centrifuged for 1 min at 5000 × g. The upper reservoir was washed twice with 500 µl washing buffer and centrifuged for 1 min at 5000 × g. 200 µl of prewarmed (70°C) elution buffer was added and the tubes were centrifuged for 1 min at 500 × g.

Randomly Amplified Polymorphic DNA (RAPD) Fingerprinting (Williams *et al.*, 1990). RAPD was carried out with some modification. The PCR mixture was composed of 10 × PCR buffer: 100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) gelatin, pH 8.3.2 mM of each dGTP, dATP, dCTP, and dTTP was added. Taq DNA polymerases: Taq DNA polymerase (5 U/µL; Sigma). Template DNA: 10 to 25 ng/µl stock solution containing good-quality, protein-free, DNA can be resuspended in high-quality sterile, deionized water or TE (Tris-EDTA) pH 8.0. RNase (20 ng per 1 ng of DNA). The following primer (OPA-02 5'TG CCGAGCTG3') was used in this study at 25 pmol/µl.

The amplifications were done in thermal cycler (PerkinElmer model 9700) programmed for the first five cycles to denature for 1 min at 94°C, anneal for 2 min at 27°C followed by primer extension for 2 min at 72°C. Then, a program for 45 cycles of 1 min denaturation at 94°C, 2 min of annealing at 32°C and 2 min primer extension at 72°C followed by a final extension period for 15 min at 72°C, was run.

Gel preparation and sample loading (Maniatis *et al*., 1982). A 0.7% agarose was prepared in 1 × TBE and mixed with 0.5 ug/ml of ethidium bromide. The gel was transferred to electrophoresis cell with 1 × TBE buffer. Each sample (20 µl) was mixed with 4 ul loading dye and loaded into the gel and 1 µl DNA marker ØX 174 Hae III was loaded into one well of the gel.

Electrophoresis and detection. 80 volts for 2 hours as 7.5 v/cm of the gel was applied. The gel was visualized using UV transilluminator and photographed by Polaroid film in Polaroid camera with 4 seconds exposure time.

Results

Characterization and identification of the isolated bacteria. Seventy-seven isolated bacterial samples were divided into three groups namely; group 1 (thirty-nine isolates were identified as *Escherichia coli*), group 2 (twenty-two isolates were identified as *Klebsiella pneumoniae*) and group 3 (sixteen isolates were identified as *Pseudomonas aeruginosa*) according to their colony morphology, colony smell, Gram stain response, shapes, pigmentation and biochemical properties.

E. coli was the most frequent bacterium isolated (50%) followed by *K. pneumoniae* (29 %) and *P. aeruginosa* (21%). Imipenem, amikacin and piperacillin/tazobactam were the most commonly used drugs for the treatment of *E. coli* with 100% effectivity. Also imipenem and amikacin were effective against *K. pneumoniae* and *P. aeruginosa* as the effectiveness of both agents on them was 100%.

Amoxicillin/clavulanate, had a lower effect against *E. coli* (72%). Nitrofurantion, cefotaxime, norfloxacin and trimethoprim/sulfamethoxazole had a low effect against *E. coli* (46%, 26%, 26% and 26% respectively). Amoxicillin/clavulanate, nitrofurantion, cefotaxime and norfloxacin had a low and similar effect on *K. pneumoniae* (27%). Cefotaxime and norfloxacin also showed low-level effectiveness against *P. aeruginosa* (50%). In addition, *P. aeruginosa* were completely resistant to nitrofurantion. Finally, trimethoprim/sulfamethoxazole had the lowest effect among the tested antibiotics with *K. pneumoniae* and *P. aeruginosa* being resistant to them.

From the antibiotic susceptibility, ten patterns were recorded (four for *E. coli*, three for *K. pneumoniae* and three for *P. aeruginosa* respectively).

For isolates of *E. coli*, the first pattern was resistant to amoxicillin/clavulanat, nitrofurantion, cefotaxime, norofloxacin, and trimethoprim/sulfamethoxazole. The second pattern was resistant to nitrofurantion, cefotaxime, norofloxacin, and trimethoprim/sulfamethoxazole. Pattern two was resistant to cefotaxime, norfloxacin, and trimethoprim/sulfamethoxazole. Finally, pattern four was susceptible to all the tested antibiotics.

The isolates of *K. pneumoniae* were distributed into three patterns. The first pattern was resistant to piperacillin /tazobactama, amoxicillin/clavulanat, nitrofurantion, cefotaxime, norfloxacin, and trimethoprim/sulfamethoxazole. Pattern two was resistant to amoxicillin/clavulanate, nitrofurantion, cefotaxime, norfloxacin, and trimethoprim/sulfamethoxazole. Pattern three was resistant to trimethoprim/sulfamethoxazole.

On the other hand, *P. aeruginosa* isolates were separated into three patterns. The first one was resistant to amikacin, piperacillin/tazobactam, nitrofurantion, cefotaxime, norofloxacin and trimethoprim/sulfamethoxazole. The second was resistant to nitrofurantion, cefotaxime, norfloxacin and trimethoprim/sulfamethoxazole. Finally pattern three was resistant to nitrofurantion and trimethoprim/ sulfamethoxazole.

RAPD-PCR analysis revealed different genotypes for all the identified bacteria. *E. coli* had a different RAPD pattern (Fig. 1), as did *K. pneumoniae* and *P. aeruginosa* (Fig. 1 and 2). RAPD technique allowed the amplification of many bands in all the isolated bacteria. There was a difference in intensity of bands within the same pattern or between the different

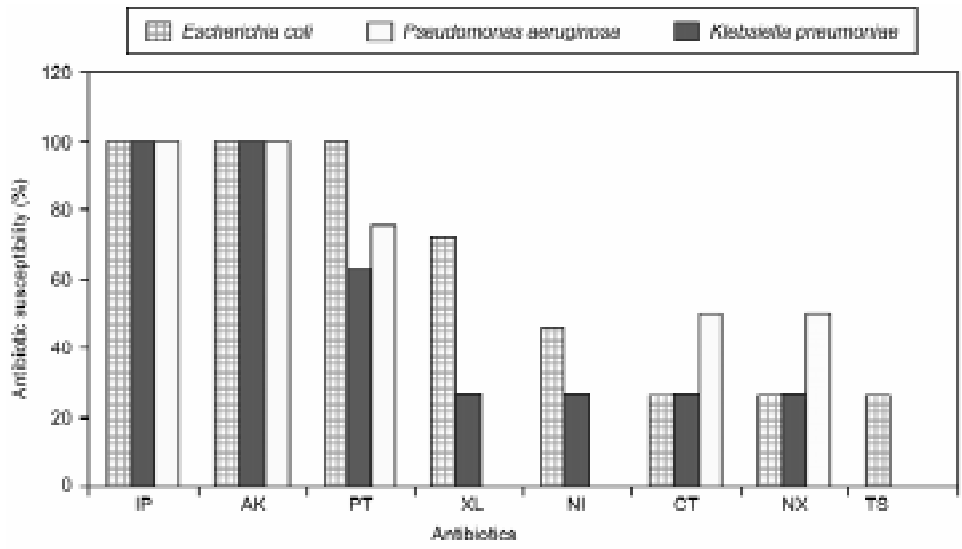

Fig. 1. Effect of different antibiotics on *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

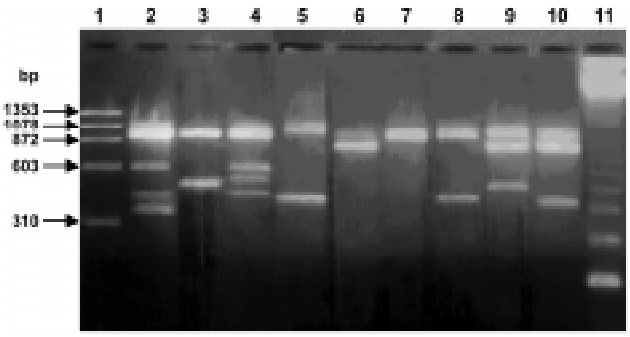

Fig. 2. Polymerase Chain Reaction-Amplified DNA of a selection of isolates of *E. coli* and *Klebsiella pneumoniae* showing random amplification of polymorphic DNA assay fingerprints, separated by gel electrophoresis and detected by ethidium bromide staining.

Lane 1: DNA molecular weight marker of *Hae III* digested phage øx174; Lanes 2 to 8 *E. coli* DNAs; Lane 9 and 10, *K. pneumoniae* DNAs; Lane 11 DNA ladders.

patterns. However, many isolates were found to be identical in genotype-displayed variability in antibiotic susceptibility pattern.

For *E. coli* isolates, the amplification of eight bands ranging in size from 280 to about 1078 bp occurred. All isolates were found to have a band of 975 bp. The four patterns resulting from the antibiotic susceptibility testing of *E. coli* were divided into seven patterns in RAPD analysis (Fig. 1).

On the other hand, eleven bands ranging in size from 360 to about 1900 bp were amplified in the isolates of *K. pneumoniae*. There was no common band in all the isolates. Three patterns of *K. pneumoniae* resulting from the antibiotic susceptibility testing were divided into five distinct patterns in RAPD method (Fig. 1 and 2).

Furthermore, nine bands ranging in size form 502 to about 1840 were amplified in the isolates of *P. aeruginosa*. All isolates were found to have a band of 1078 bp. Three patterns of *P. aeruginosa* resulting from the antibiotic susceptibility testing were divided into five distinct patterns in RAPD analysis (Fig. 2).

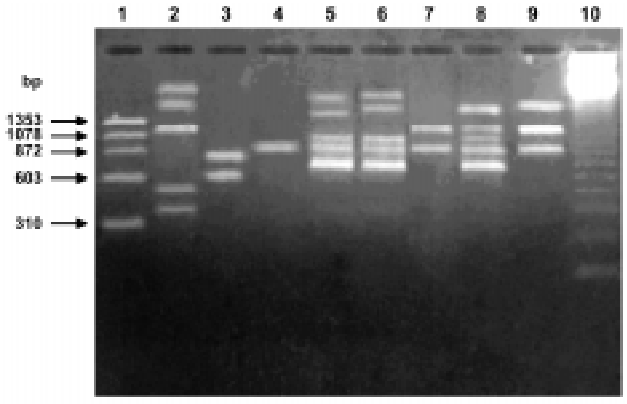

Fig. 3. Polymerase Chain Reaction-Amplified DNA of a selection of isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showing random amplification of polymorphic DNA assay fingerprints, separated by gel electrophoresis and detected by ethidium bromide staining.

Lane 1: DNA molecular weight marker of *Hae III* digested phage øx174; Lanes 2 to 4 *K. pneumoniae* DNAs; Lane 5 and 9 *P. aeruginosa* DNAs; Lane 10 DNA ladders.

The RAPD patterns of different isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa* from Urology and Nephrology Center, Mansoura University may suggest that those isolates constitute a clonal lineage.

Discussion

Most of the clinical isolates of *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in this study were susceptible to imipenem and amikacin with 100% susceptibility rate and the degree of resistance to the other tested multiple antibiotics varied according to the antibiotics. Foda (2008) recorded that meronem and amikacin were highly active towards *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Pseudomonas aeruginosa.* He also reported that the susceptibility rate of urinary isolates was 76.19% for meronem followed by amikacin (70.27%). Das *et al*. (2006) showed that the susceptibility rate of urinary isolates was the highest for amikacin (87.2%).

The simplicity and wide applicability of the RAPD method is dependent on the use of short nucleotide primers, which are not related to known DNA sequences of the target organism. They are designed within constraints including (i) a length of not less than nine nucleotide residues, (ii) a GC content of >50% and (iii) a lack of palindromic sequences (William *et al.*, 1990). These primers used in PCRs have been able to efficiently detect DNA polymorphisms and identify interstrain variations in an increasing number of species (Bingen *et al.*, 1993a; 1993b). Genetic mapping and determination of the degree of relatedness between strains have been performed with validation by ribotyping (Williams *et al.*, 1993). The banding pattern derived in this process allows the identification of similar strains by a method significantly less complicated and time consuming than ribotyping. When directly compared in the analysis of bacterial sample outbreak in a maternity unit, RAPD and ribotyping were equivalent in their abilities to discriminate between strains (Bingen *et al.*, 1993a). It is of paramount importance that reaction conditions, including DNA template concentration, annealing temperature, and other PCR mixture concentration are strictly standardized to avoid artifactural variation in RAPD patterns (Ellsworth *et al.*, 1993).

While RAPD gives information regarding similarity between isolates, the application of PCR based techniques has a revolutionary impact on the diagnosis of infectious diseases. The most commonly used

molecular genetic fingerprinting technique by RAPD revealed more genetic differences among avian bacterial strain than amplified fragment length polymorphism (AFLP) analysis (Gomes *et al.*, 2005). In recent years with the advent of molecular DNA techniques, several arbitrary primer based RAPD-PCR techniques have been used for delineating the bacteria according to their genetic relatedness (Muzurier and Wernas, 1992; Eisen *et al.*, 1995; Lin *et al.*, 1996).

Earlier researchers were of the opinion that RAPD was the best method for detecting genetic differences with respect to its speed and ability to type a wide variety of bacterial species and suggested it would be an increasingly useful molecular epidemiologic tool. In the past, dendrogram-based analysis of the RAPD profiles of various bacteria allowed understanding the genetic relationship between isolates grouped into several clusters. These phylogenetic studies successfully showed the predominance of a single epidemic strain that was transmitted between hosts and its persistence over a period of time (Gomes *et al.*, 2005).

In this study the four patterns resulting from the antibiotic susceptibility testing of *E. coli* were divided into seven patterns in the DNA method. There is a difference in intensity of bands within the same pattern or between the different patterns, *i.e.* DNA method identified additional heterogeneity among the related strains. Seven patterns of *E. coli* generated by DNA based method differed by the presence or absences of one or two single DNA fragment when compared one with another. Sometimes smearing is observed when multiple DNA fragments, which differ slightly in length, are visible.

Eisen *et al.* (1995) studied multi-resistant *Klebsiella pneumoniae* strains by typing the isolates phenotypically and with random amplified polymorphic DNA analysis (RAPD) and plasmid analysis and they showed the predominance of a single epidemic strain that was transmitted between patients in the Newborn Services Unit. Lopes *et al.* (2005) found that 26 RAPD genotypes among studied 30 *K. pneumoniae* and they demonstrated the high discriminatory power of RAPD. RAPD analysis in their research indicated that pathogenic *K. pneumoniae* strains comprise a genetically high variable group of organisms. In this study, three patterns of *K. pnuomoniae* resulting from antibiotic susceptibility testing were divided into five patterns in the RAPD analysis. Lai *et al.* (2000) reported that a pathogenic *K. pneumoniae* strain was highly heterogeneous, based on the distribution of different nucleotide sequences. The high number of serotypes in this species (Orskov and Orskov, 1984) could ahlso explain the relevant degree of genetic diversity highlighted by RAPD.

Kerr *et al.* (1995) applied RAPD to 10 cases of pneumoniae associated with sputum culture of *Pseudomonas aeruginosa* and they suggested that a single strain of *P. aeruginosa*, isolated from 10 ICU patients, was responsible for this outbreak of pneumonia. Nazik *et al.* (2007) studied the typing of the *Pseudomonas aeruginosa* isolates recovered from cystic fibrosis (CF) patients by random amplified polymorphic DNA (RAPD)-PCR and to determine the antibiotic susceptibility of these strains. Their study revealed that most the *P. aeruginosa* isolates with dissimilar colony morphology or antibiotic susceptibility isolated from these CF patients were of the same genotype, but colonization or infection with only one genotype is, however, not a rule. These results were also recorded in other earlier studies (Sener *et al.*, 2001; Horrevorts *et al.*, 1990).

In this study, three patterns of *P. aeruginosa* resulting from antibiotic susceptibility testing were divided into five patterns in the DNA method, that is the DNA method identified additional heterogeneity among the related strains. Khalifa *et al.* (2010) reported that genotyping of *Pseudomonas aeruginosa* isolated from clinical samples showed 83 RAPD types and they also recorded that the isolates showing the same serotype could show different genotypes. In addition, by using RAPD, Trautmann *et al.* (2006) showed that isolates of *Pseudomonas aeruginosa* from patients showed a similar distribution of genotypes.

In conclusion, in this study, epidemiological typing of 39 *Escherichia coli*, 22 *Klebsiella pneumoniae* and 16 *Pseudomonas aeruginosa* clinical samples was carried out by phenotypic and molecular methods. RAPD technique detected genetic heterogeneity in different strains of the studied bacteria. This can be useful to understand the distribution of these pathogens in nosocomial infections.

Literature

Belkum A. 1994. DNA fingerprinting of medically important microorganisms by use of PCR. *Clin. Micribiol. Rev.* 7: 174–184.

Bingen E.H., C. Boissinot, P. Desjardins, H. Cave, N. Brahimi, N. Lambert-Zechovsky, E. Denamur, P. Blot and J. Elion. 1993a. Arbitrarily primed polymerase chain reaction provides rapid differentiation of *Proteus mirabilis* isolates from a pediatric hospital. *J. Clin. Microbiol.* 31: 1055–1059.

Bingen E.H., H. Cave, Y. Aujard, N. Lambert-Zechovsky, J. Elion and E. Denamur. 1993b. Molecular analysis of multiply recurrent meningitis due to *Escherichia coli* K1 in an infant. *Clin. Infect. Dis.* 16: 82–85.

Bosi C., A. Davin-Regli, C. Bornet, M. Mallea, J. Pages and C. Bollet. 1999. Most *Enterobacter aerogenes* strains in France belong to a prevalent clone. *J. Clin. Microbiol*. 37: 2165–2169.

Brenner D.J. 1986. Family I. Enterobacteriaceae. In: Bergey's Manual of Systematic Bacteriology (Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (eds). Williams and Wilkins, Baltimore.

Centers for Disease Control (CDC). 1983. Nosocomial infection surveillance 1980 1982. CDC Surveillance Summaries, 32 (455): 155.

Chit L.P. and C.Y. Chew. 1993. Recent advances in the typing of *Pseudomonas aeruginosa*. *J. Hosp. Infect.* 24: 175–181.

Das R.N, T.S. Chandrashekhar, H.S. Joshi, M. Gurung, N. Shrestha and P.G. Shivananda. 2006. Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal. *Singapore Med. J.* 47(4): 281–285.

Eisen D., E.G. Russeli, M. Tymms, E.J. Roper, M.L. Grayson and J. Turnidge. 1995. Random Amplified Polymorphic DNA and Plasmid Analyses Used in Investigation of an Outbreak of Multiresistant *Klebsiella pneumoniae*. *J. Clin. Microbiol.* 33: 713–717.

Ellsworth D.L., K.D. Rittenhouse and R.L. Honeycutt. 1993. Artifactual variation in randomly amplified polymorphic DNA banding patterns. *Bio-Techniques* 14: 214–217.

Foda A. 2008. Ph.D. Thesis. Zagazig University. Zagazig. Egypt.

Gales A.C., R.N. Jones, K.A. Gordon, H.S. Soder, W.W. Wike, M.L. Beach, M.A., Pfaller, M.V. Doern and the SENTRY Study Group (Latin America). 2000. Activity and spectrum of 22 antimicrobial agent tested against urinary tract infection pathogens in hospitalized patients in Latin America: reported from the second year of the SENTRY Antimicrobial Surveillance Program (1998). *J. Antimicrob. Microb. Chemotherapy* 45: 295–303.

Gomes A.R., L. Muniyappa, G. Krishnappa, V.V.S. Suryanaryna, S. Isloor, B. Parakash and P.G. Hugar. 2005**.** Genotypic Characterization of Avian *Escherichia coli* by Random Amplification of Polymorphic DNA. *International J. Poultry Science* 3: 378–381.

Gori A., F. Espinasse, A. Deplano, C. Nonhoff, M.H. Nicolas and M.J. Struelens. 1996. Comparison of PFGE and RAPD for typing extended-spectrum beta-lactamase producing *Klebsiella pneumoniae. J. Clin. Microbiol.* 10: 2448–2453.

Gupta N., S. Kundra, A. Sharma and V. Gautam. 2007. Antimicrobial susceptibility of uropathogens in India. *J. Infect. Dis. Antimicrob. Agents*. 24:13–18.

Hilton A.C. and C.W. Penn. 1998. Comparison of ribotyping and arbitrarily primed PCR for molecular typing of *Salmonella enterica* and relationships between strains on the basis of these molecular markers. *J. Appl. Microbiol.* 85: 933–940.

Horrevorts A.M., J. Borst and R.J. Puyk. 1990. Ecology of *Pseudomonas aeruginosa* in patient with cystic fibrosis. *J. Med. Microbial.* 31:119–124.

Khalifa A. B., H. Vu-Thien, C. Pourcel, M. Khedher, M. Mastouri and D. Moissenet. 2010. Phenotypic and genotypic (randomly amplified polymorphic analysis and multiple locus variable-number tandem-repeat analysis) characterization of 96 clinical isolates of *Pseudomonas aeruginosa* in the F. Bourguiba (Monastir, Tunisia). *Pathologie Biologie* 58: 84–88.

Kerr J.R., J.E. Moore, M.D. Curran, R. Grahm, C.H. Webb, K.G. Lowry, P.G. Murphy, T.S. Wilson and W.P. Ferguson. 1995. Investigation of a nosocomial outbreak of *Pseudomonas aeruginosa* pneumonia in an intensive care unit by random amplification of polymorphic DNA assay. *J. Hospital Infection.* 30: 125–131.

Lai Y.C., S.L. Yang, H.L. Peng and H.Y. Chang. 2000. Identification of genes present specifically in a virulent strain of *Klebsiella pneumoniae. Infect. Control.* 68: 7149–7151.

Lin A.W., M.A. Usera, T.J. Barrettand and R.A. Goldsby. 1996. Amplification of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella enteritidis*. *J. Clin. Microbial.* 34: 870–876.

Lopes, A.C., J.F. Rodrigues and M. A. Júnior. 2005. Molecular typing of *Klebsiella pneumoniae* isolates form public hospitals in Recife, Brazil. *Microbiological Research.* 160: 37–46.

Lübeck P.S. and J. Hoorfar. 2003. PCR technology and applications to zoonotic food-borne bacterial pathogens. In: PCR detection of microbial pathogens (Methods in molecular biology) Volume 216, (Sachse, K. and Frey, J. (Editors), Humana press.

Maniatis T., E.F. Fritsch and J. Sambrook. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor. New York.

Muzurier S.I. and K. Wernas. 1992. Typing of *Listeria* strains by random amplification of polymorphic DNA. *Res. Microbiol.* 143: 499–505.

National Committee for Clinical Laboratory Standards (NCCLS). 2006. Interpretation of antibiotic sensitivity according to guidelines of NCCLS.

Nazik H., B. Ongen, B. Erturan and M. Salcioglu. 2007. Genotype and antibiotic susceptibility patterns of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophila* isolated from cystic fibrosis patients. *Jpn. J. Infect. Dis.* 60: 82–86.

Orskov I. and F. Orskov. 1984. Serotyping of *Klebsiella pneumoniae*. *Methods Microbiol.* 14: 143–164.

Ørskov F. 1986a. Genus I. *Escherichia*. In: Bergey's Manual of Systematic Bacteriology (Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (eds). Williams and Wilkins, Baltimore.

Ørskov I. 1986b. Genus V. *Klebsiella*. In: Bergey's Manual of Systematic Bacteriology (Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J. and Williams, S.T. (eds). Williams and Wilkins, Baltimore.

Palleroni N.J. 1986. Family I. Pseudomonadaceae. In: Bergey's Manual of systematic Bacteriology (Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (eds). Williams and Wilkins, Baltimore.

Patton T.G., S. Katz, R.J. Sobieski and S.S. Crupper. 2001. Genotyping of clinical *Serratia marcescens* isolate: a comparison of PCR-based methods. *FEMS Microbiol. Lett.* 194: 19–25.

Sener B., O. Koseoglu and U. Ozcelik. 2001. Epidemiology of chronic *Pseudomonas aeruginosa* infectin in cystic fibrosis. *Int. J. Med. Microbial*. 291: 387–393.

Trautmann M., C. Bauer, C. Schumann, P. Hahn, M. Höher, M. Haller and P.M. Lepper. 2006. Common RAPD pattern of *Pseudomonas aeruginosa* from patients and Tap water un a medical intensive care unit. *Int. J. Hyg. Environ. Health*. 209: 325–331.

Turck M. and W. Stamm. 1981. Nosocomial infections of the urinary tract. *Am. J. Med.* 70: 651.

Welsh J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acid Res.* 18: 7213–7218.

Williams J.G.K., A. R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990**.** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531–6535.

Williams J.G.K., M.K. Hanafey, J.A. Rafalski and S.V. Tingey. 1993. Genetic analysis using random amplified polymorphic DNA markers. *Methods Enzymol.* 218: 704–744.

Yuksel S., B. Ozturk, A. Kavaz, Z.B. Ozcakar, B. Acar, H. Guriz, D. Aysev, M., Ekim and F. Yalcinkaya. 2006. Antibiotic resistance or urinary tract pathogen and evaluation of empirical treatment in Turkish children with urinary tract infections. *Int. J. Antimicro. Agents.* 5: 413–416.