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Molecular evaluation of antibiotic resistance prevalence in *Pseudomonas aeroginosa* isolated from cockroaches in Southwest Iran

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Pseudomonas aeruginosa **can cause infection in the hospitals. This microorganism is ubiquitous within the environment and is particularly isolated from moist areas such as water and soil. The aim of this study was to determine the hospital cockroaches as the main factor for antibiotic-Resistant *P. aeruginosa* infections transmission and also to determine antibiogram pattern of *P. aeruginosa*. Also, the pattern of antibiotic resistance showed that a total 14 (11.2%) of 125 samples were contaminated with *P. aeruginosa* isolated from cockroaches in hospitals using molecular sieve of polymerase chain reaction (PCR). The overall susceptible of isolated *P. aeruginosa* strains to antimicrobial agents showed that Amikacin 14 (100%), Ciprofloxacim 14 (100%), Gentamycin 14 (100%), have more susceptibility, respectively. The prevalence of *P. aeruginosa* in cockroaches' hospitals is high and as a potential factor in transmission of *P. aeruginosa.***

Key words: *Pseudomonas aeruginosa*, antibiotic resistance, cockroaches.

INTRODUCTION

Pseudomonas aeruginosa play an important role in hospital intensive care units, causing a wide spectrum of nosocomial infections (Strom and Lory, 1986; Gomila et al., 2006). *P. aeruginosa* is an aerobic, nonsporulating Gram-negative, motile bacterium (Römling et al., 1994). *P. aeruginosa* causes infection in immune depressed subjects or in those with faulty homeostasis mechanisms (Struelen et al., 1993). *P. aeruginosa* potential pathogens have been isolated from cockroaches collected from hospitals and have proven that cockroaches carry a large flora of pathogenic bacteria (Babalola et al., 2007). The source of the pathogenic versatility of *P. aeruginosa* is undoubtedly its unique genome. Sequencing of this genome was achieved in 2000 (Canduela et al., 2006). For the year 1991 to 1992, *Pseudomonas* species were the most commonly identified etiologic agents causing dermatitis, conjunctivitis, or otitis in humans following recreational water exposures where water pH or free residual chlorine levels failed to meet Centers for Disease Control and Prevention (CDC) guidelines for public spas and hot tubs (Malathi et al., 2006; Tsuchizaki et al., 2006). *P. aeruginosa* can be a constituent of the bacterial flora of the intestines of laboratory rodents, especially mice. *P. aeruginosa* strains are resistant to β-lactams, aminoglycosides, and quinolones (Buckingham-Meyer et al., 2007; Willcox et al., 2008).

P. aeruginosa is among the most feared pathogens associated with nosocomial infection, especially among mechanically ventilated patients (Trautmann et al., 2005). *P. aeruginosa* is the most common pathogen responsible

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Table 1. TEM, SHV, and CTX-M primers of *P. aeruginosa*.

Gene	Sequence	PCR product (bp)
Tem	Tem-F: 5'-TCCGCTCATGAGACAATAACC-3' Tem-R: 3'- ATAATACCGCACCACATAGCAG-5'	296
Ctx-M	Ctx-F: 5'-TCTTCCAGAATAAGGAATCCC-3' Ctx-R: 3'-CCGTTTCCGCTATTACAAAC-5'	909
Shv	Shv-F: 5'-TACCATGAGCGATAACAGCG-3' Shv-R: 3'-GATTTGCTGATTTCGCTCGG-5'	450

for both acute respiratory infections in ventilated or immunocompromised patients and chronic respiratory infections in cystic fibrosis patients (Johnson et al., 2007; Rello et al., 1997). Flagella and pili, the motile surface appendages of *P. aeruginosa* are responsible for bacteria motility and progression towards epithelial contact (Mahenthiralingam et al., 1996; Rello et al., 1996). Upon cell contact, the type III secretion system, a major virulence determinant, is activated. Four effectors proteins are known: ExoY, ExoS, ExoT, and ExoU and all participate, at varying levels, in the cytotoxicity of *P. aeruginosa* leading to invasion and dissemination of *P. aeruginosa* (Morfin-Otero et al., 2009). The aim of this research was to determine the prevalence *P. aeruginosa* in cockroaches from hospital in Chaharmahal VA Bakhtiar, Iran using polymerase chain reaction (PCR). The study also tried to specify the pattern of antibiotic resistance in summer of 2011.

MATERIALS AND METHODS

Sample collection

This empirical study was done on 125 cockroaches collected from 6 hospitals located in Chaharmahal Va Bakhtiari province (Hajar and Ayatollah Kashani Hospitals in Shahrekord city, Shohada Hospitak in Farsan city, Imam Javad in Naghan city, Imam Reza in Lordagan city, and Valiasr in Boroujen city). The collection of samples was done using manual and sticky trap methods from hospital kitchens. Then, samples were transmitted to the laboratory of Biotechnology Research Center using separate sterile tube to prevent any contamination mixing of the samples. *Klebsiella pneumoniae* ATCC 700603 (Genekam Biotechnology AG, Germany) and *Escherichia coli* ATCC 25922 was used as positive and negative control, respectively.

Picking up the cultivation of susceptibility

Antimicrobial susceptibility profiles were determined by the dilution method on Mueller-Hinton agar, according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (Knowle et al., 1992). The antimicrobial agents tested included trimethoprim, sulfamethoxazol, ceftiazidime, ceftriaxon, imipeneme, topramycin, amikacin, ciprofloxacin and gentamcin. CLSI breakpoints were used for minimum inhibitory concentration (MIC) interpretation (Empel et al., 2007). The results were interpreted after 24 h of incubation at 37°C, as sensitive, intermediately sensitive, and resistant according to the zone of diameter around each antibiotic disk.

PCR assay

In order to confirm the presence of *P. aeruginosa* TEM, SHV, and CTX-M genes, PCR test was performed. The primers used for genes amplification are as shown in Table 1.

Amplification reaction was carried out in a total volume of 25 µl, consisting of 1 µM of each primers, 2 mM $Mgcl_2$, 200 µM dNTP, 5 µl of 10X PCR buffer, 1 U of Taq DNA polymerase (Fermentas, Germany) and 1 µg of template DNA. Thermal PCR conditions consisted of 5 min at 95°C and then 30 cycles initial temperature of 94°C, temperature of 58 and 72°C connector at each end for 1 min and final extension was for 5 min at 72°C. The amplified products were analyzed in 1.5% agarose gel. Electrode buffer was TBE (Tris-base 10.8 g 89 mM, Boric acid 5.5 g 2 mM, EDTA (pH 8.0) 4 ml of 0.5 M EDTA (pH8.0) combined all components in sufficient H_2O and stir to dissolve). Gels were stained with ethidium bromide. Aliquots of 10 µl of PCR products were applied to the gel. Constant voltage of 80 for 20 min was used for products separation. After electrophoresis images were obtained in UVItec documentation systems (UK).

Statistical analysis

The numbers of cockroaches presenting airsacculitis and the prevalence of re-isolation of *P. aeruginosa* from the swap were analyzed by the chi-square test using the SPSS17 (SPSS Inc. Chicago, IL, USA) software. The probability level for significance was $p \leq 0.05$.

RESULTS

The quality of extracted DNA from samples was examined by electrophoretic analysis through a 1% agarose gel. *P. aeruginosa* was recovered from 14 cockroaches of 125 (11.2%). The percentage of resistant isolates was as follows: 14.28% to ceftriaxon, 13.3% to ceftazidim, 26.66% to sulfamethoxazol and percentage of susceptible isolates was as follows: 100% to tobramycin, imipenem, gentamycin, ciprofloxacin and amikacin. Also, antibiotic resistance pattern is as shown in Table 2.

The TEM gene of *P. aeruginosa* was success fully

Table 2. Antibiotic resistance pattern of *P. aeruginosa*.

Antimicrobial Agent	Resister	Half Resister	Stark
Trimethoprim sulfamethoxazol (sxt)	26.66	21.42	46.66
Amikacin (AN_{30})	0	0	100
Ciprofloxacim (CP_5)	0	0	100
Gentamycin (GM_{10})	0	0	100
Ceftriaxon (CRO_{30})	14.28	57.14	14.28
Imipeneme (IPM_{10})	0	0	100
Ceftiazidime (CAZ_{30})	13.3	28.57	53.33
Topramycin (TOB_{10})	0	0	100

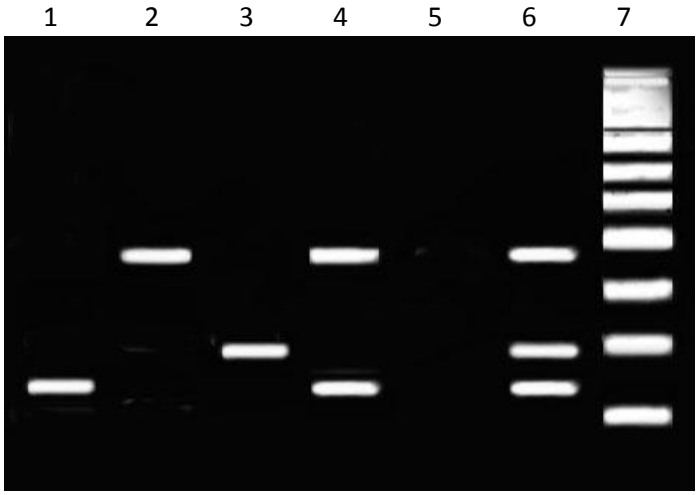

Figure 1. An agarose gel stained with ethidium bromide, for detection of TEM, SHV and CTX-M genes in P. *aeruginosa*. Lines 1, 2 and 3 are positive tests for TEM, CTX-M and SHV, respectively. Line 4 is positive test for TEM and CTX-M. Lines 5 and 6 are negative and positive controls, respectively. Line 7 is 1 kb DNA ladder (Fermentas, Germany).

amplified with the TEM-F and TEM-R primers. Also, agarose gel electrophoresis of the PCR amplified products is as shown in Figure 1. Out of 14 *P. aeruginosa,* 14 samples (100%), 1 sample (7/14%) and 3 samples (21/42%) are positive for TEM, SHV and CTX-M genes by PCR, respectively. The most active agents to treat infection caused by *P. aeruginosa* were amikasin, ciprofloxacin, gentamycin, imipenem and tobramycin.

DISCUSSION

Pseudomonas consists of 5 types that include *Pseudomonas spasya*, *Pseudomonas maltvfyiya*, *Pseudomonas financial*, *Pseudomonas psvdvmalyy* and *P. aeruginosa*. *P. aeruginosa* is one of the most important nosocomial pathogen and is strongly involved in severe and often fatal infections in patients with cystic fibrosis, burns, ocular diseases, pneumonia, and other immunosuppressive illnesses (5,1). Although *P. aeruginosa* is a nonfermentative aerobe, it can grow under anaerobic conditions using nitrate as an electron receptor. Its ability to survive in a wide range of environmental conditions is partially explained by its versatile nutritional abilities and its ability to resist high concentrations of common antibiotics (Aumeran et al., 2007). *P. aeruginosa* is intrinsically resistant to the most commonly used antibiotics. Antibiotic resistance is achieved through a combination of restricted antibiotic uptake through the outer membrane and a variety of energy-dependent mechanisms (Presteri et al., 2007). Preincubation with antibiotics has been demonstrated to have a number of effects on *P. aeruginosa* including induction of a biofilm form of growth, improved heat and osmotic stress response, changes to hydrophobicity, and reduced bacterial adherence (Panagea et al., 2005).

According to a study in Iranian patients with cystic fibrosis (CF), the antibiograms of the isolates showed 100% sensitivity to imipenem and collistin followed by ciprofloxacin (90.5%), ceftazidime and tobramycin (85.7%), amikacin, piperacillin, gentamycin (62%), and carbenicillin (43%). In other study, MIC determination for amikacin showed a 100% sensitivity as compared to the disk test where 81% sensitivity was observed (Ferrante and Scortichini, 2009; Huson and Bryant, 2006). In other hand, according to Giamarellos-Bourboulis et al. (2006) study, *P. aeroginosa* was resistant to ciprofloxacin (27.1%), ceftazidime (15.7%), cefepime (2.9%), imipeneme (67.1%), and piperacilin (14.3%), whereas in our study *P. aeroginosa* was resistant to sulfamethoxazol (26.66%), ceftriaxon (14.28%), ceftiazidime (13.3%), imepenem (0%), amikacin (0%), ciprofloxacim (0%), gentamycin (0%), and topramycin (0%).

Genomic DNA was extracted and the PCR were performed using specific primers for TEM, SHV and CTX-M genes. The results of the resent study showed that TEM gene is present in all of the isolated *P. aeruginosa* and SHV and CTX-M genes present in some isolates.

Conclusion

Conclusively, as stated earlier, this study showed that isolated *P. aeruginosa* of cockroaches from hospital have more resistance to Sulfamethoxazol rate of 26.66%, rather than other studies. Amikacin, ciprofloxacin, gentamcin, and topramycin seem to be the only antimicrobial agent which showed 100% sensitivity and may be used as the drug of choice for treating multidrug resistant *P. aeruginosa* infections. Further, the regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern of *P. aeruginosa* and formulation of definite antibiotic policy may be helpful for reducing the incidence of *P. aeruginosa* infection.

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