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Antibiotic sensitivity and genotyping of *Acinetobacter baumannii* isolated from clinical and environmental samples of burn hospital of Tehran using MLVA method

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ABSTRACT

Acinetobacter baumannii has emerged as an important hospital pathogen worldwide especially in the burn ward. The aim of this study is to determine the pattern of antibiotic susceptibility and genotyping of *A. baumannii* isolated from clinical and environmental samples of Shahid Motahari hospital in Tehran using MLVA method. In this study, 173 clinical and 28 environmental isolates of *A. baumannii* were collected from Shahid Motahari hospital within a 9-month period (2018-2019). The isolates were confirmed by biochemical and molecular tests with *OXA-51* primer. Antibiotic sensitivity was performed by the disc diffusion method according CLSI M100-S21 guidelines. MLVA-PCR was performed with six STR markers including *Abaum-3530*, *Abaum-3002*, *Abaum-2240*, *Abaum-1988*, *Abaum-826*, and *Abaum-2396*. Out of 201 tested strains for antibiotic sensitivity, 127 (63.2%) and 35 (17.5%) of isolates of the strains were multidrug-resistant (MDR) and extensively drug-resistant (XRD). Microsatellite typing of 201 *A. baumannii* isolates showed 197 genotypes in four clusters. The Hunter-Gaston diversity index (HGDI) of six markers (STRs) for all isolates was 0.9169. The progressive increase in *A. baumannii* infections and antibiotic resistance in hospitals demands some measures for rapid description of the typing of isolates and identification of sources of infection. Our results indicated that MLVA a method based on PCR was more effective for typing of clinical and environmental strains of *A. baumannii*. These findings highlight the importance of international resistance against different antibiotics as well as molecular epidemiological control of *A. baumannii* isolates with XDR and MDR characteristics.

1. Introduction

Burn wound surface provides an encouraging niche for bacterial colonization and proliferation (Sharma et al., 2014). This infection may be originate from the endogenous and exogenous sources (Erol et al., 2004). *Acinetobacter* spp. remain as normal flora of the human skin, can be simply transmitted, and as well, it is remain viable in the hospital environment (Towner, 2009). Bacterial infections with multidrug resistance in burn patients have high mortality (Alaghehbandan et al., 2012).

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Acinetobacter baumannii is an opportunistic pathogen which is involved in development of hospital or nosocomial infections especially in burn wards (Azimi et al., 2015). *A. baumannii* is the second cause of multidrug resistance those producing hospital infections in burn patients in Iran (Farshadzadeh et al., 2015). It is considered as one of the life-threatening microorganisms which are resistant to antimicrobial agents because of its considerable clinical features especially in recent years and its ability in acquiring drug resistance (Ardebili et al., 2012).

Several typing methods have been considered for tracking the epidemiology of *A. baumannii* infection worldwide. The methods which have been described in previous studies include pulsed-field gel electrophoresis (PFGE) (Chang et al., 2013), multi-locus sequence typing (MLST) (Bartual et al., 2005; Diancourt et al., 2010), amplified fragment length polymorphism (AFLP) fingerprinting (Van Dessel et al., 2004), and analysis Variable-Number Tandem-Repeat (VNTR) (Pourcel et al., 2011).

Genotyping methods provided the information related to prevalence of diseases. For example, the genetic diversity of clones, their temporal and spatial distribution, their consequences in endemic or epidemic an occurrence, source of infection, as well as the number of affected patients, where these preventive and controlling measures are essential (Villalón et al., 2015).

Multiple-locus variable-number tandem-repeat analysis (MLVA) has demonstrated to be a rapid, valid, and cost-effective typing technique for some bacterial species (Petersen et al., 2011; Stietz et al., 2013). In a MLVA test, a pre-described set of VNTRs marker is evaluated, and a code regarding to the number of repeats at each locus can be identified for separate strains (Pourcel et al., 2011). In a study, MLVA analysis showed that sequence type 2 [ST-2] was dominance in *A. baumannii* isolated from Spanish hospitals. In addition, three new clones ST-79, ST-80, and ST-81 were also discovered (Villalón et al., 2011).

The aim of present study was genotyping *A. baumannii* strains isolated from clinical and environmental samples of burn hospital in Tehran using MLVA method.

2. Materials and Methods

2.1. Bacterial isolates

Overall, 201 no repetitive strains of *A. baumannii* including 173 clinical isolates obtained from burn wounds and 28 environmental isolates were collected from February 2018 to November 2019 from Motahari hospital, Tehran. All isolates were identified using by chemical and microbiological tests by API20NE kit (bioMerieux, France). Then, final confirmation was done using PCR method through the specific primer *blaOXA-51* (Sohrabi et al., 2012).

2.2. Antibiotic profiles

A. baumannii isolates were tested for sensitivity to ceftazidime (30 µg), cephotaxim (30 µg), cefepime (30µg), gentamicin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), trimethoprim-sulfamethoxazole (23.75+1.25 µg), aztreonam (30 µg), and piperacillin-tazobactam (30 µg) by the Kirby-Bauer disk diffusion method (CLSI 2018). All of the antibiotic discs were prepared from MAST Co. (Mast Diagnostics, UK). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 bacterial isolates were used as control (Vahaboglu et al., 2006).

2.3. MLVA-PCR

Genomic DNA was extracted by the boiling procedure. The MLVA typing scheme was performed as defined previously in a study by Hauck et al. (Hauck et al., 2012). The six loci including *Abaum 826*, *Abaum 2396*, *Abaum 1988*, *Abaum 2240*, *Abaum 3002*, and *Abaum 3530* primers were used (Hauck et al., 2012). PCRs were conducted in a 25 µL final volume containing 0.5 unit of *Taq* DNA polymerase, reaction buffer (1X),

1.5 mM of $MgCl_2$, 200 µM of dNTP, 1 µl of each primers (20 pM), and 1 µl of DNA (3 ng) in thermal cycler device (BIORAD, C1000) in 30 cycles. PCR programs were as follows: initial denaturation for 10 min at 94°C, followed by 30 cycles of denaturation for 35 sec at 94°C, annealing for 30 sec at 50°C for *Abaum0845* and *Abaum0826* and at 55°C for the rest loci, extension for 50 sec at 72°C, and a final extension for 7 min at 72°C. Then, 10 µl of PCR product was loaded on agarose gel 1.5%. The size of the amplicons was measured by GeneTools software from cinnagen Company.

The copy number = number of repetitions/size of offsets - the size of the replicated region. The relationship between different genotypes was analyzed through comparing the allele profiles via BioNumeric V.7.0 software.

3. Results

The antibiotic-resistant profile displayed that the percentage of resistance to cefotaxime, cefepime, gentamicin, ciprofloxacin, amikacin, imipenem, meropenem, trimethoprim-sulfamethoxazole, aztreonam, ceftazidime, and piperacillin-tazobactam have been 87.5%, 83.5%, 81%, 91 %, 89%, 83.5%, 85.5%, 93.5%, 92.5%, 83%, and 86.5% respectively. Out of the 201 tested strains, 127 (63.2%) and 35 (17.5%) of the strains were multidrug-resistant (MDR) and extensively drug-resistant (XDR) respectively. The results of dendrogram analysis (Fig. 1) and graphic representation of the minimum tree spanning (Fig. 2) indicate the allele profile of 201 *A. baumannii* isolates. Microsatellite typing of 201 *A. baumannii* isolates (173 clinical and 28 environmental isolates) indicated 197 genotypes in four clusters. Among them, 4 types of double clonal complexes are observed: three clonal complexes including two clinical isolates and one clonal complex including one clinical isolated and one environmental isolate. Hunter-Gaston diversity index (HGDI) of the STR assay technique based on Simpson's diversity index was determined based on each of the STR marketers, which ranged from 0.601 to 0.973. Further, the differentiation power of this method considering all of the six STR markers utilized was 0.916. There were no significant relationship was observed between genotypes and antibiotic sensitivity (Pvalue<0.05).

4. Discussion

A. baumannii nosocomial infection is an increasing and currently it is considered as a worldwide threat (Babakir-Mina et al., 2017; Lima et al., 2019). *A. baumannii* infection especially in the burn wound patients has been the major cause of morbidity (Alp et al., 2012; Atilla et al., 2015). The MLVA-PCR can identify the clonal closely in *A. baumannii* and as well decrease the distribution of these isolates among burn cases (Azimi et al., 2016). Our results of the drug sensitivity profile indicated that this bacterium was resistant to different drugs as follows: cefotaxime (87.5%), cefepime (83.5%), gentamicin (81%), ciprofloxacin (91%), amikacin (89%), imipenem (83.5%), meropenem (85.5%), trimethoprim-sulfamethoxazole (93.5%), aztreonam (92.5%), ceftazidime (83%), and piperacillin- tazobactam (86.5%). Hosseini Jazani et al. found the resistance of the *A. baumannii* isolates to the following antibiotics: piperacillin 88.9%, gentamicin 70.8%, ofloxacin 95.8%, ceftizoxime 75%, cefatolin 60.4%, ticarcillin 93.7%, kanamycin 95.8%, imipenem 14.6%, amikacin 52%, cotrimoxazole 79.1%, cefazolin 100%, and carbenicillin 93.7% (Hosseini Jazani et al. 2009). Also, Ardabili et al. (2012) found the following resistances: cotrimoxazole 58%, tobramycin 62%, gentamicin 86%, imipenem 93%, amikacin 94%, ticarcillin 95%, ceftazidime 98%, aztreonam 98%, piperacillin and tazobactam 96%, and cefotaxime 96% (Ardebili et al., 2012). Nourbakhsh et al (2018) reported the resistances rates as following: gentamicin (69.6%), ciprofloxacin (97.2%), amikacin (47.2%), imipenem (44%), meropenem (67.2%), trimethoprim-sulfamethoxazole (59%), and ceftazidime (88.3%) (Nourbakhsh et al., 2018). Shakibaii et al.

indicated the resistance rate to imipenem, ciprofloxacin, piperacillin-tazobactam, amikacin, cefepime and piperacillin have been 73.3%, 66%, 93.3%, 53.3%, 93.3%, and 100%, respectively (Shakibaii et al., 2012). Different profile of antibiotic resistance rates in various studies can be associated to clinical specimen types or geographic area of the test (Aliakbarzade et al., 2014).

Microsatellite typing of 201 isolates of *A. baumannii* showed 197 individual genotypes in four clusters. The HGDI of STR assay technique based on Simpson's diversity index for each of the STR markers was determined, which ranged from 0.601 to 0.973. The differentiation power of this method in current study was 0.9169. Azimi et al. (2016) investigated the genotyping of 50 carbapenem-resistant isolates of *A. baumannii* isolated from burn patients via MLVA method. Their results showed five different typing algorithms with Simpson's index diversity of 0.63 (Azimi et al., 2016). Najarpirayeh and Karamstaji (2019) investigated the genotyping of 89 carbapenem-resistant strains of *A. baumannii* isolated from burn patients from two hospital centers in Tehran through MLVA method. They found five different typing algorithms with Hunter-Gaston diversity index of 0.99 (Najarpirayeh and Karamstaji 2019). In their study, presence of *Abaum 0845* in MLVA caused a great diversity, such that it was not possible to make clusters of the isolates, thereby highly complicating interpretation of its results (Najarpirayeh and Karamstaji 2019).

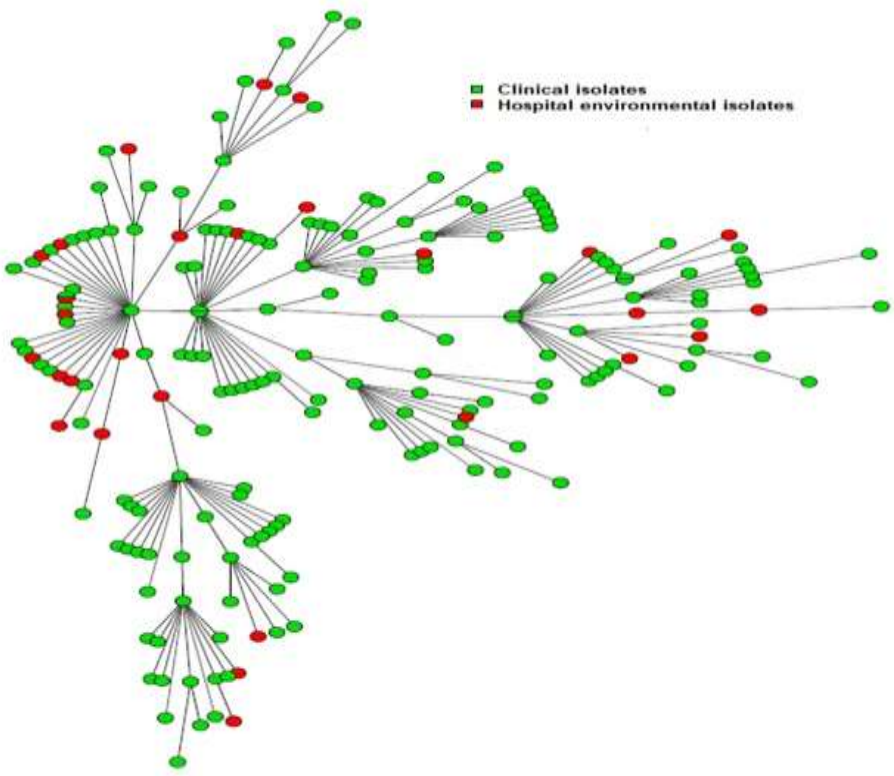

Fig 1. Minimum Spanning tree genetic diversity of 201 isolates of Acinetobacter baumannii in MLVA

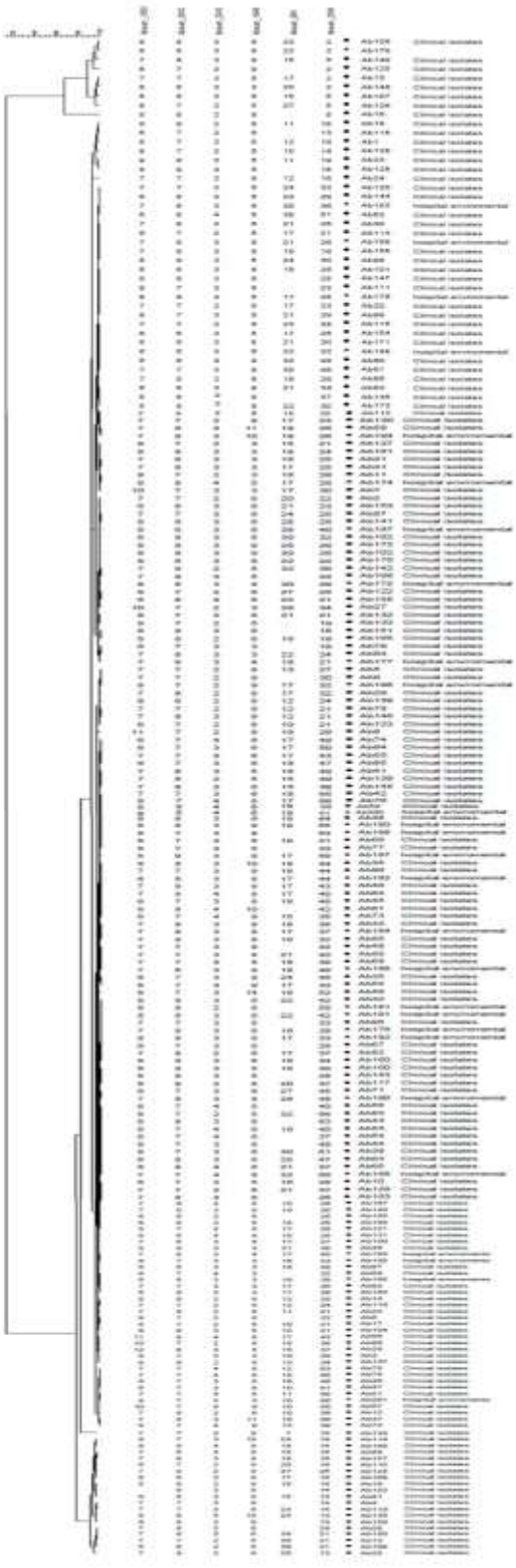

Fig 2. Dendrogram Analysis Results of Genetic Diversity of 201 Acinetobacter baumannii Isolates in MLVA Technique

Therefore, analysis of this marker VNTR in the study by Najarpirayeh and Karamstaji (2019) as well as the study by Bahador et al. (Bahador et al., 2015) was excluded from the study. In addition, the diversity associated with *Abaum 0845* has been reported in another study performed in Spain (Villalón et al., 2011). Tuan Anh et al. (2017) performed MLVA analysis on 160 strains of *A. baumannii* from the clinical samples collected from Vietnam (Tuan Anh et al., 2016). Out of this number of isolates, based on the VNTR findings in their study, 107 types were isolated into five clusters (Tuan Anh et al., 2016). Farshadzadeh et al. (2015) deals with typing of 92 no repetitive strains of *A. baumannii* isolated from burn wound infection via MLVA technique. They found 56 types of specific genotypes in six clusters as well as 53 individual genotypes (Farshadzadeh et al., 2015). Hu et al. (2013) in China investigated 122 isolates of *A. baumannii* with eight markers of VNTR (Hu et al., 2013). They observed that MLVA-7 marker had a HGDI=0.985 compared to the seven markers designed by Pourcel et al. (Pourcel et al., 2011; Hu et al., 2013). The results of the study by Saffari et al. showed that the Abaum 0017 (allele 21) and Abaum0826 (allele 20) had the maximum power in differentiating 64 isolates of *A. baumannii*. In this study, cluster analysis showed nine clonal complexes as well as 28 individual genotypes (Saffari et al., 2017). The results of analysis of four markers of VNTR for 59 *A. baumannii* isolates in the study by Hauck et al. showed 11 clusters plus 13 individual genotypes (Hauck et al. 2012). Rahimi et al. analyzed MLVA for 80 clinical isolates. They found that every strain had a unique type of MLVA (MT), and all isolates were divided into 14 separate clusters (Rahimi et al., 2018). The reasons of the difference between the results of the present study and the other researchers' findings can be due to type of strain, geographical region, source of samples, and type of the microsatellite marker used. MLVA is a fast, economical, and easy to use typing method which enjoys excellent replicability and differentiation power, enabling interlaboratory transfer.

Therefore, the molecular typing such as MLVA-PCR and susceptibility patterns of *A. baumannii* in our burn center should be conducted regularly in order to control the antibiotic resistant pathogens in burn infection patients.

Conclusion

The results of the present study confirmed development of antibiotic resistant *A. baumannii* strains and their resulting healthcare problems in Iran. Further, molecular typing of the isolates isolated from clinical and environmental samples of *A. baumannii* isolates offers new insight into important public health and epidemiological problems including the sources and ways of transfer, identifying pathogenic or drug-resistant strains, and the genetic relationship between the strains.

Refereces

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