Frequency of efflux pump genes mediating ciprofloxacin and antiseptic resistance in methicillin-resistant Staphylococcus aureus isolates

Sepideh Hassanzadeh a, Rahil Mashhadi b, Masoud Yousefi c, Emran Askari a, Maryam Saniei b, Mohammad Reza Pourmand a,*

a Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
b Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran
c Department of Microbiology, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran

A R T I C L E   I N F O

Article history:
Received 18 July 2016
Received in revised form
14 August 2017
Accepted 16 August 2017
Available online 18 August 2017

Keywords:
Methicillin-resistant Staphylococcus aureus
Efflux pump genes
Ciprofloxacin resistance

A B S T R A C T

Efflux pumps are well known as a key role to fluoroquinolone resistance in methicillin-resistant Staphylococcus aureus (MRSA). In this study, among 60 clinical MRSA isolates, 42 isolates (70%) were resistant to ciprofloxacin. MRSA were isolated to detect efflux genes including norA, norB, norC, mepA, sepA, mdeA, qacA/B and smr. Isolates subjected to PCR detection and DNA sequence analysis for these genes. PCR detection showed that 42 isolates (70%) contained at least one efflux pump gene. Among ciprofloxacin-resistant isolates, mdeA and qacA/B genes were found with the highest (61.7%) and lowest (3.3%) frequency, respectively. We also observed that the highest minimum inhibitory concentrations of ciprofloxacin in the presence of mdeA+mepA+norA-C+sepA+smr combination. This type of combination may have the greatest impact on resistance to ciprofloxacin. Finally, compared to previous studies, our study demonstrates that prevalence of ciprofloxacin resistance has been increasing among MRSA clinical isolates.

© 2017 Published by Elsevier Ltd.

1. Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is a major concern in health-care and community settings [1]. MRSA strains are resistant to nearly all beta-lactam antibiotics by producing an alternative penicillin-binding protein known as PBP2a [2]. This protein is encoded by the mecA gene and has a low affinity to many beta-lactam antibiotics. Furthermore, these strains often show resistance to a wide range of antibiotics [3]. The use of fluoroquinolone for the effective infectious therapy is limited by presence of fluoroquinolone resistance [4,5].

There are two mechanisms causing resistance to fluoroquinolone. The first one is attributed to mutations occurring in the quinolone-resistance determining region (QRDR) of topoisomerase IV encoded by grlA/grlB and DNA gyrase encoded by gyrA/gyrB; these mutations decrease the affinity of the drug. The other mechanism is mediated by efflux pumps which is less recognized [6].

Recently, several efflux pumps have been identified for S. aureus including efflux pumps encoded by chromosome or plasmids. The efflux pumps norA, norB, norC, mdeA, sepA, mepA, sdrM and lmrS are encoded by chromosome while qacA/B, qacG, qacH, qacJ and smr are plasmid-encoded [7,8]. Efflux pumps could be specialized for specific substrate or mobilized a wide varieties of different antibiotic classes [9,10]. For example, Vali et al. reported in their study that overexpression of norB convey a pattern of resistance to fluoroquinolones, tetracyclines, disinfectants, and dyes. It is worthwhile to mention that the same pattern of resistance observed with overexpression of mepA [11].

Qac proteins are responsible for biocides’ resistance, could be found in approximately 40% of MRSA strains in Europe and Asia [12]. Furthermore, Huet et al. demonstrated a biocide resistance phenotype in clinical isolates with the overexpression of efflux pump genes [13]. The aim of this study was to evaluate the frequency of efflux pump genes mediating ciprofloxacin and antiseptic resistance in MRSA isolates in Tehran, Iran.

* Corresponding author. Department of Pathobiology, School of Public Health and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
E-mail address: mpourmand@tums.ac.ir (M.R. Pourmand).
2. Materials and methods

2.1. Bacterial strains

From December 2012 to April 2014, we collected 120 S. aureus isolates from three hospitals affiliated with Tehran University of Medical Sciences.

2.2. Bacterial identification

All clinical isolates were identified as S. aureus by conventional biochemical tests [14]. MRSA strains were identified by three different methods, cefoxitin disc diffusion (30 μg, MAST Diagnostics, UK) on Mueller-Hinton agar (MHA), all cefoxitin resistant isolates subjected to evaluate minimum inhibitory concentration (MIC) of oxacillin by MIC Test Strip (Lioflichem, Italy) technique according to clinical and laboratory standards institute (CLSI) guideline [15] and detection of the mecA gene by PCR. Control strains for methicillin-resistant and -susceptible S. aureus were COL and ATCC 8325-4 strains, respectively.

2.3. Antimicrobial susceptibility testing

For MRSA isolates, the antibiotic susceptibility testing was performed by disc diffusion method against these antibiotics: clindamycin (2 μg), ciprofloxacin (5 μg), erythromycin (15 μg), trimethoprim (30 μg), vancomycin (30 μg), teicoplanin (30 μg), doxycycline (30 μg), and nitrofurantoin (300 μg) (all purchased from MAST Diagnostics, UK). The results interpreted according to CLSI guidelines [15].

All isolates were subjected to evaluate MIC of ciprofloxacin by MIC Test Strip (Lioflichem, Italy) according to CLSI guidelines [15].

2.4. PCR amplification

All MRSA isolates tested for presence of six chromosomal and two plasmid encoded genes. Total genomic DNA extracted by VioGene kit (Viogene, Taiwan) based on manufacturer’s instructions and used as a template for PCR reaction. Specific primers used for amplification of norA, norB, norC, sepA, mepA, mdeA, qacA/B and smr genes (Table 1) [16–18]. Amplification conditions were as follows: DNA was denatured at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C (norA), 62 °C (norB, norC) and 61 °C (sepA, mepA, mdeA) for 45–55 s and extension at 72 °C for 1 min, followed by a step of final extension at 72 °C for 5 min. The PCR reactions for genes qacA/B and smr conducted under the following conditions: DNA was denatured at 95 °C for 1 min, followed by 30 cycles of denaturation at 95 °C each for 1 min, annealing at 58 °C (qacA/B, smr) for 45 s and extension at 72 °C for 1 min, followed by a step of final extension at 72 °C for 5 min. The amplification products were electrophoresed in 1% agarose gel at 100 V for 1 h, stained with KBC (0.5 μg/ml) (Kawsar, Iran), and photographed under UV light.

All results were confirmed by at least two independent experiments. Each PCR products were sequenced by Takapouzist Company and deposited at GenBank (accession number KJ909760.1).

2.5. Statistical analysis

Statistical analysis performed using SPSS v.17.0 statistics software. Pearson’s chi-square test used to assess inter-group significance. In addition, the association of antibiotics resistance and presence of efflux pump genes investigated.

3. Results

3.1. MRSA isolates detection

By cefoxitin disc diffusion method and PCR of the mecA gene, 60 (50%) isolates were found to be MRSA. Isolates were obtained from different clinical specimens including blood cultures (19 isolates), wound swabs (22 isolates), urine (15 isolates) and sputum (4 isolates) specimens. The MIC of oxacillin was determined by strip found 55 (45.83%) isolates with MIC between 8 and 16 μg/ml. The rest five MRSA strains were only identified by cefoxitin disc diffusion and had an MIC of oxacillin between 0.125 and 0.5 μg/ml.

3.2. Susceptibility data

MRSA isolates showed high resistance to clindamycin (63.3%), and erythromycin (58.3%). There was a moderate resistance to doxycycline and trimethoprim (28.3% for both). Ten isolates (16.7%) were immediately resistant to teicoplanin. All isolates were susceptible to vancomycin and nitrofurantoin. The details of antimicrobial susceptibility pattern are shown in Table 2. According to MIC results, 42 isolates (70%) were resistant to ciprofloxacin (Table 3).

3.3. Molecular detection of efflux pump genes

The presence of efflux pump genes was assessed by PCR. Efflux pump genes were significantly more common in ciprofloxacin-resistant isolates compared to ciprofloxacin-susceptible ones. According to the statistical test, presences of norA, norB, mepA, mdeA and sepA genes were significant correlation with ciprofloxacin resistant (P-value< 0.05).

The highest and lowest frequency was related to mdeA (61.7%) and qacA/B (3.3%) genes, respectively. In addition, the frequency of mepA, norA, norB, norC, sepA and smr were 60%, 41.7%, 41.7%, 35% and 30%, respectively. Among ciprofloxacin-susceptible MRSA isolates, mepA gene was the most common. The highest MIC of ciprofloxacin resistant was seen in the presence of mdeA+mepA+norA-C+sepA+smr combination (Table 4).

4. Discussion

This study shows that the prevalence of ciprofloxacin resistance is 70%, while in other studies in Tehran; it has been reported from 29% to 99% [19–22]. This is the first study to our knowledge to report detection of efflux pump genes using clinical MRSA isolates in this region. We demonstrates the frequency of efflux pump genes
alone or along to

These isolates have one or two ef

ported 90% resistance to cipro

parC

and spread of cipro

cin. In other hand, the presence of

ux pumps genes in

oxacin resistant.

smr

e

e

ux pumps genes that most of them was

ux pumps extrude QAC and dye compounds

Multidrug efflux pumps qacA, qacB and smr that encoded by plasmid are widely spread among MRSA. Several countries have been reporting prevalence of qac genes in more than 40% of MRSA isolates [27]. In contrast, a recent report from Canada indicated that the distribution of qacA/B and smr was 2% and 7%, respectively [28].

Studies on clinical isolates in Asia have been shown the prevalence ranging up to 73% and 32% for the qacA/B and smr genes, respectively [29]. In European studies we also found different prevalence values; qacA/B ranging 8.3%, 15%, 44%, smr ranging 4%, 6%, 44% [11,30]. In our study among 60 MRSA clinical isolates from Tehran hospitals, we found 3.3% occurrence of qacA/B and 30% smr genes.

One of the limitations of our study was that we only investigated the frequency of ef genes but not their expression. Many studies have shown an increased expression of ciprofloxacin efflux genes particularly among MRSA isolates compared to methicillin-resistant ones [31]. The overexpression of efflux pump genes in MRSA and MSSA strains were 50% and 49%, respectively [6]. In S. aureus, the chromosomally encoded MDR efflux pumps norA, norB, norC, mepA and mdeA are widely present in different strains.

5. Conclusions

In conclusion, 70% MRSA isolates were phenotypically resistant to ciprofloxacin. In other hand, the presence of mdeA+mepA+norA-C+sepA+smr combination may cause ciprofloxacin resistant. Further studies are necessary to determine the expression of these efflux pumps genes.

Acknowledgments

We kindly thank Mr. Peyman Avakh and Ms. Sanaz Dehbashi at the Department of Pathobiology for contribution to the project. The authors also wish to express their gratitude to Research Council of Tehran University of Medical Sciences (TUMS), Iran, for financial support [Grant number 20105].

Conflict of interest

The authors declare that they have no conflict of interest.

References


