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Original Research Article

Prevalence and molecular characterization of plasmid-mediated beta-lactamase genes among nosocomial Staphylococcus aureus isolated in Taiwan

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Abstract

Purpose: *To analyze the drug susceptibility phenotypes and the patterns of plasmid-mediated β-lactamase genes among nosocomial Staphylococcus aureus drug resistance isolates in Taiwan.*
Methods: *The antibiotic susceptibilities of 617 clinical Staphylococcus aureus isolates collected from 2005 - 2009 from Chiayi Christian Hospital (Chiayi, Taiwan) were examined in vitro against 8 antimicrobial agents using agar diffusion method. Among the clinical isolates, 114 strains of methicillin-sensitive Staphylococcus aureus and 45 strains of methicillin-resistant Staphylococcus aureus (MRSA) isolates were selected for plasmid profile analysis. The patterns of β-lactamase genes presented in plasmids were investigated by polymerase chain reaction analysis.*
Results: *Most test strains were resistant to multiple antibiotics, particularly for the traditional agents such as ampicillin, penicillin, cephalexin and kanamycin. Plasmid profile analysis revealed that up to 36 % of the clinical strains harbored plasmids and were able to develop multi-drug resistant. Among them, most of the isolates harbored at least one plasmid (range 1 – 7) with a size range of 2.3 to 23 Kb. Among the several types of β-lactamases, blaTEM was the most prevalent.*
Conclusion: *The results obtained from this study can serve as a valuable reference for the future control for clinical antibiotic resistant strains and more thorough discussions on resistance mechanisms.*

Keywords: *Staphylococcus aureus, Antibiotic susceptibility, Nosocomial pathogens, Plasmid profile, β-lactamases*

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INTRODUCTION

The widespread use of antibiotics has given rise to the development of resistant strains. Relatively common strains of infectious bacterium, methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA) have acquired resistance to various antimicrobial agents in Taiwan, thus complicating the treatment of nosocomial infections [1-3]. Dissemination of antibiotic resistant genes by horizontal transfer has led to the rapid emergence of antibiotic resistance among clinical isolates. Given positive selection pressure for antibiotics, the nature of the bacterial colony can be transformed from sensitivity resistance in an astonishingly short time. Nonpathogenic bacteria have been found to harbor resistant genes which can be shared with pathogens [4,5].

Studies have revealed the extensive role played by agents, called plasmids, and related components, known as transposons and integrons [6]. Plasmids often carry drug resistance genes and can sometimes confer resistance to a number of different drugs. Strains resistant to these antibiotics mediated by β-lactamases have now spread worldwide.

β-lactamases contain several types , in which TEM, OXA and Z are resistant genes usually located on plasmids [7]. β-lactamase typing has been established as an important addendum to the characterization and identification of clinical antibiotic resistance pathogens and is routinely used in many laboratories [8].

Analyses of plasmids and their related genetic information have provided important insights into the reasons and mechanisms behind the emerging antibiotic resistance problem. The present study analyzes plasmid DNA profiles and patterns of β-lactamase genes, including TEM, OXA and Z to demonstrate the genetic profiles and their corresponding drug susceptibility phenotypes among *Staphylococcus aureus* drug resistance isolates.

EXPERIMENTAL

Collection of strains

A total of 617 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA) were obtained between 2005 and 2009 from Chiayi Christian Hospital (Chiayi, Taiwan). All of the clinical isolates were authorized by Chiayi Christian Hospital. Strains were routinely grown overnight at 37 ℃ on Luria-Bertani broth under aerobic condition.

Antibiotic susceptibility test

Susceptibility to antibiotic drugs was determined on Muller Hinton agar, according to the Clinical and Laboratory Standards Institute guidelines [9]. The plate were impregnated with different antibiotics, including Erythromycin (30 ug), Ampicillin (50 ug), Gentamicin (15 ug), Tetracycline (15 μg), Penicillin G (30 μg), Cephalexin (30 μg), Amoxicillin (30 μg), Kanamycin (15 μg), Streptomycin (15 μg), Trimethoprim/Sulfamethoxazole (30 μg), and Clindamycin (15 μg), The bacteria were screened in the plates and incubated at 37 ^{o}C for 16 h. All susceptibility tests were carried out in duplicate and repeated twice if discordant initial results were obtained.

Plasmid DNA extraction and transformation

Plasmid DNA was isolated using the alkaline lysis method [10]. The isolated plasmids were transfected in competent DH5α cells by heat shock plated on Ampicillin (50 μg/mL) plates and incubated overnight at 37 ^{o}C.

Polymerase chain reaction (PCR) amplification

Plasmid DNA isolated from *S. aureus* was used as a template for polymerase chain reaction (PCR) of β-lactamase gene. The oligonucleotide primers were designed on the basis of the nucleotide sequence in GenBank (http://www.ncbi.nlm.nih.gov/genbank). The PCR primers for OXA (bla_{OXA}), ampC (bla_{ampC}), TEM (*bla*TEM), and bla_{Z} genes were designed using our previously developed software, SPD (http://bio.kuas.edu.tw/ma-spd/seqin.jsp) [11]. The primer sets used are listed in Table 1.

PCR technique was performed in a 25 uL reaction mixture containing 150 uL of DNA template, 0.4 umol/L of each primer, 200 umol/L each of the four nucleotides triphosphates, 2 mM $MgCl_2$, 500 mM KCl, 10 mM Tris (pH 8.0) and 1 u of *Taq* DNA polymerase. Samples were subjected to the following thermocycling process (Pertin Elmer 2400): 94 ^{o}C for 5 min, followed by 30 cycles of 94 ^{o}C for 1 min, 60 ^{o}C for 1 min and 72 ^{o}C for 1 min. A final extension step at 72 ^{o}C was continued for another 7 min. A tube containing the reaction mixture and sterile water was included in all reactions as a negative control. Amplicons were electrophoresed on a 1 % agarose gel (Sigma-Aldrich) in Tris-Borate-EDTA buffer after staining with ethidium bromide (1 μg/mL).

Table 1: Primers used in this study

Primer	Nucleotide sequence (5' – 3')	PCR targets	Tm ℃	Size (bp)
bla_{oxa273}	F:TgAgCACCATAAggCAACCA R:TTgggCTAAATggAAgCgTTT	bla_{oxa}	55.0	311
$bla_{Z\ 514}$	F:gTggTAAggAAgTAAAATTTAA R:AAgCATATgTTATTgCTTgAC	bla_{Z}	56.0	561
bla_{TEM}	F:AggAAgAgTATgATTCAACA R:CTCgTCgTTTggTATggC	bla_{TEM}	55.0	535

Gels were visualized and photographed under ultraviolet illumination using GelDoc-XR apparatus (Bio-Rad).

DNA sequencing

The DNA sequences of bla_{TEM} PCR amplicons from all positive isolates were determined following cycle sequencing reactions. Nucleotide sequences were analyzed and compared to those available in the GenBank database by use of the BLAST computer program (NCBI) and the FASTA program.

RESULTS

The drug susceptibility profiles were examined by agar-screening test. A total of 617 clinical isolates exhibited resistance to one or more of the following antibiotic agents: penicillin g, ampicillin, cephalexin, streptomycin, kanamycin, gentamycin, erythromycin and trimethoprim/ sulfamethoxazole. Among the antibiotic resistant pathogens, the resistance phenotypes of isolates collected from 2005 - 2007 revealed drug resistance to antibiotics as follows: penicillin g (90 %), ampicillin (77 %), cephalexin (75 %), streptomycin (59 %), kanamycin (77 %), gentamycin (54 %), erythromycin (55 %), and trimethoprim/sulfamethoxazole (98 %). The isolates collected from 2008 - 2009 revealed drug resistance to antibiotics as follows: penicillin g (75 %), ampicillin (76 %), cephalexin (55 %), streptomycin (34 %), kanamycin (67 %), gentamycin (44 %), erythromycin (54 %), and trimethoprim/sulfamethoxazole (23 %) as shown in Figure 1.

Among the 617 isolates, 114 MSSA isolates and 45 MRSA isolates were selected for plasmid analysis. It was found that 35 out of 114 MSSA isolates carried one or more plasmids, as opposed to 28 out of 45 MRSA isolates. Most of the isolates harbored more than one plasmid (range 1 – 7) with a size between 2.3 to 23.1 Kb. Based on the harbored plasmid, the isolates are classified into several profiles (Table 2). All of the isolated plasmids were transformed into the *E. coli* DH5α host cells. The plasmid profiles of transforms were identical with their original isolate donors (Fig. 2).

Compared with the donors, the transforms harbored partially resistant genes which are confirmed by the resistance to antibiotics, including streptomycin, kanamycin, and gentamycin, tetracycline and trimethoprim/ sulfamethoxazole.

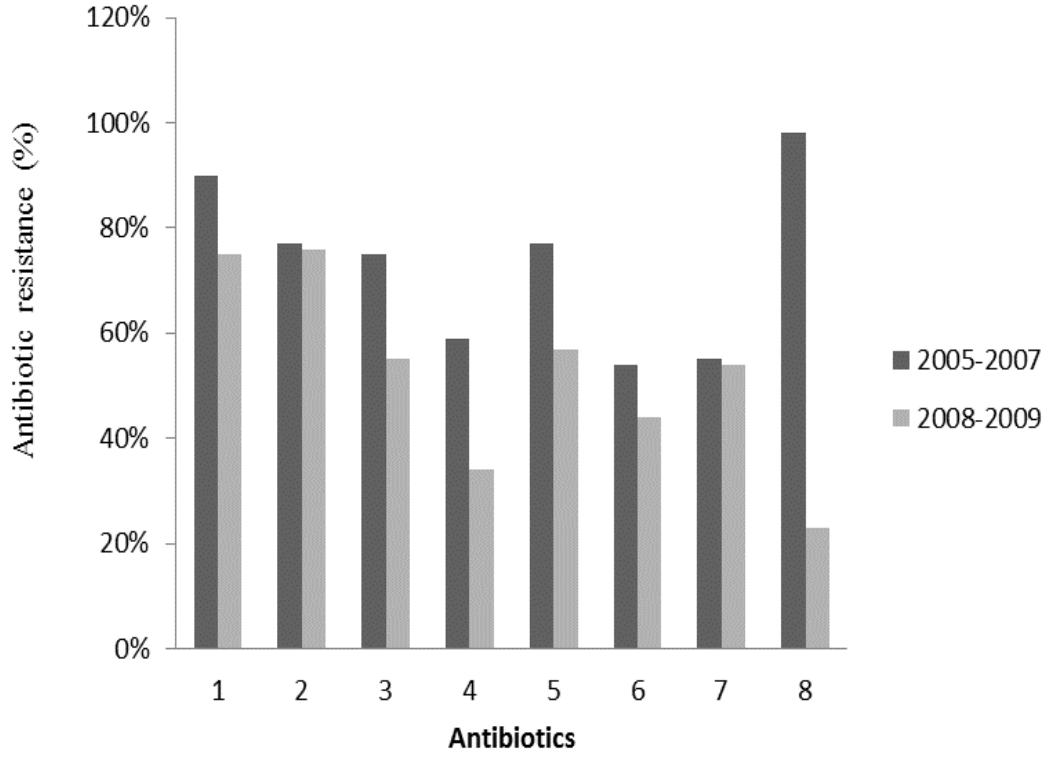

Figure 1: Distribution of antimicrobial susceptibility of the clinical antibiotic resistant pathogens.
1. Penicillin G; 2. Ampicillin; 3. Cephalexin; 4. Streptomycin; 5. Kanamycin; 6. Gentamycin; 7. Erythromycin; 8. Trimethoprim/Sulfamethoxazole

Table 2: Plasmid profiles of the clinical antibiotic-resistant pathogens

Strain	Profile	Isolate (N)	Plasmid number	Isolate (%)	Plasmid size (Kb)
Methicillin sensitive *S. aureus*	I	4	4	13	23.1, 9.4, 4.0, 2.3
	II	8	4	27	23.1, 5.5, 4.4, 2.3
	III	11	4	37	23.1, 9.4, 5.5, 4.0
	IV	7	3	23	23.1, 9.4, 4.4
Methicillin resistant *S. aureus*	I	17	1	61	23.1
	II	5	4	18	23.1, 9.4, 6.6
	III	3	7	11	23.1, 9.4, 7.0, 6.6, 4.4, 3.0, 2.3
	IV	2	6	7	23.1, 9.4, 4.4, 4.0, 2.8, 2.3
	V	1	3	4	23.1, 9.4, 5.5

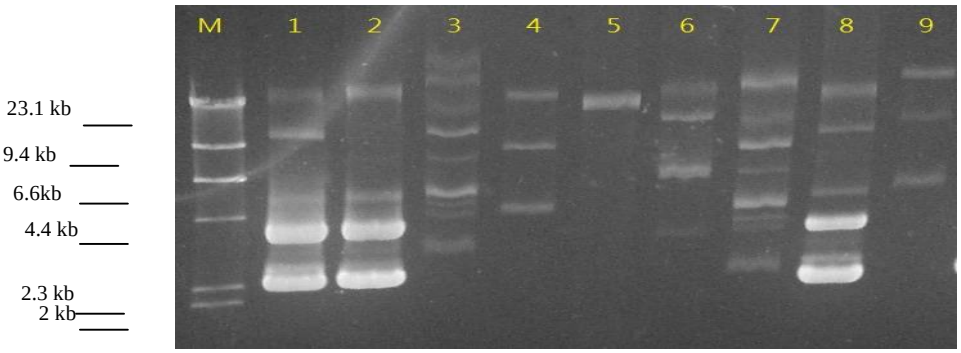

Figure 2: Plasmid profiles of the plasmid transforms. Lane M: Lambda DNA / *Hind* III Marker; Lane 1-4: Methicillin Sensitive *Staphylococcus aureus* profiles I, II, III, and IV, respectively; Lane 5-9: Methicillin Resistant *Staphylococcus aureus* profiles I, II, III, IV, and V, respectively

The results showed that 9 transforms of MSSA donors and 13 transforms of MRSA donors presented different levels of antibiotic susceptibility and profiles. Transforms from each resistance profile were randomly selected for further genetic characterization, with 22 plasmid transforms subjected to genetic analysis. Since most of the test isolates revealed a higher resistance toward β-lactams, the genetic analysis was focused on the β-lactamase gene. In PCR analysis of the β-lactamase gene, the bla_{TEM} gene was found in all test strains. However, none of the test strains were found to carry other test β-lactamase genes. All the bla_{TEM} amplicons were subjected to DNA sequencing. The obtained sequences were compared with TEM-1 gene sequence (AY560328.1, AY263331.1) from the NCBI website with 98 % homology to relevance.

DISCUSSION

The study mainly focuses on clinical drug resistance strains of Methicillin-sensitive *S. aureus* and Methicillin-resistant *S. aureus*, which were isolated from a teaching hospital in Taiwan. The antibiotic susceptibility results showed that about 75 – 97 % of the isolates were resistant to the β-lactam antibiotics, 54 – 77 % to the aminoglycoside antibiotics, 46 – 98 % to the other test antibiotics, and over 94 % of the test isolates exhibited multiple drug resistance. Because all samples were obtained from a single hospital, the high percentage of multiple drug-resistant strains might be due to the horizontal transfer of genetic elements between bacterial strains. More strains showed resistance toβ-lactam than to other types of antibiotics, consistent with previous findings [11,12].

Comparing the antibiotic susceptibility of the test strains isolated in different years, some antibiotics, including Cephalexin, Streptomycin, Kanamycin and Trimethoprim/Sulfamethoxazole, showed a relative decline in the proportion of resistance, highly consistent with the reduced use of antibiotic treatment (data not shown). Thus, routine investigation of antibiotic susceptibility patterns for nosocomial pathogens is important to provide information for optimized treatment guidelines and new therapeutic alternatives [13].

According to antibiotic resistance gene analysis, 100 % of transforms possessed bla_{TEM} gene. Compared with our previous study [14], this shows that the rate of plasmids conjugated with antibiotic resistance gene has increased. The results suggest that multiple drug resistant pathogens harboring antibiotic resistant plasmids can be easily dispersed among other bacteria, resulting in the rapid spread of antibiotic resistance genes [6]. For the antibiotic susceptibility test, among the antibiotic

aminoglycosides class, the overall resistance ratio of the test strains of streptomycin, kanamycin and gentamycin were respectively 59 – 34 %, 77 – 67 % and 54 – 44 %. Aminoglycoside resistance has been widely observed in the plasmids of antibiotic resistance pathogens [15,16]. Resistance to sulfonamides and trimethoprim/sulfamethoxazole showed the highest radio (98 %) among the test antibiotics in 2005 – 2007, possibly because the antibiotics treatment and pathogens were isolated from the same hospital [17,18]. Among the plasmid transforms, 100 % of the transforms exhibited multiple drug resistance, suggesting that plasmid conjugation increases antibiotic resistance and the prevalence rate [19].

β-lactamase producing pathogens are being increasingly identified around the world and are prevalent in several countries in the Asia-Pacific region [7]. In PCR detection of β-lactamase genotypes, the only prevalent genotype was found to be bla_{TEM} (100 %). None of the test samples was carriers of bla_{OXA} and bla_{Z} genes. The differences in the results obtained between the present study and other reports might be due to geographical differences, difference in infection types, the excessive use of antibiotics in Taiwan, or antibiotic therapy practices. The results of this study revealed that about 100 % of the test strains carried bla_{TEM} gene, which is consistent with the results obtained from the sequence analysis by sequence alignment with gene *TEM*-1 (AY560328.1) on the NCBI website. Previous studies noted that most strains harboring bla_{TEM} gene exhibited a high degree of resistance to β-lactam antibiotics and the *TEM*-1 drug resistance gene is among the more common types of *TEM* drug-resistant pathogens in China and Taiwan [20].

CONCLUSION

Most of the test strains are highly resistant to the β-lactam antibiotics and all the strains harbor the bla_{TEM} gene. The bla_{TEM} PCR analysis from plasmid gene profile indicate a relative positive correlation to the phenotype. This suggests that a rapid screening method based on bla_{TEM} gene might be valuable. The findings of this study can serve as a valuable reference for future control on clinical drug resistant strains and more thorough discussions on resistance mechanisms.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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