

Molecular Detection of *MepA* Gene from *Staphylococcus Aureus* Isolated from Cases of Tonsillitis

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Abstract

Introduction:

Bacterial tonsillitis defined as an infection of the tract of upper respiratory tract. This infection infects adolescents and children. *S. aureus* is the pathogen that occurs in tonsillitis etiology due to resistance to antimicrobials in the tonsil tissues. Tonsillectomy that presents in tonsillitis cases after treatment failures by therapy with antibiotics.

Design of the study:

To complete this research, we procured 17 surgically removed tonsils from individuals who had a previous history of tonsillitis. When we tested the tonsils for *Staphylococcus aureus*, we sent them to a lab for microbiological testing to look for the particular microorganism. All isolates were tested by PCR for the presence or absence of *mepA* gene.

Results:

From 17 patients who were 12 years old on average, 15 isolates were obtained. *mepA* gene was present in all isolates. Most isolates were resistant to many antibiotics.

Conclusions:

The isolates of *S. aureus* were resistant to most antibiotics and all have *mepA* gene.

Introduction

Infection in the palatine tonsils called tonsillitis that infects children, as well as young adults.¹ Many studies explained recurrent tonsillitis etiology, that remains a controversial topic. But acute tonsillitis caused by single microbial species, it suggested polymicrobial infection is which causes recurrent tonsillitis.² Also, chronic tonsillitis caused by other microorganisms.¹ Bacterial biofilm that defined that important factor involved in resistance for antibiotic treatment and infections chronicity. For this reason, these infections give negative impact on significant burden on public health patients' also life quality. Bacterial biofilms that have important role in recurrent

respiratory tract infections, that include chronic disease for tonsillar, also that found in the children tissue infected with chronic infections in tonsils.³ *S. aureus* produce by beta lactamase promoting resistance for penicillin in tonsils microbiota.

Many studies show antibiotic therapy failure because underestimation for resistant microorganisms,¹ that explain by low concentrations for antibiotic in tonsillar tissue, combined with bacteria presence which producing resistance patterns for antibiotic or protective enzyme, where persistence in this site that because bacterium presence in tonsil tissue. Tonsillar surface presents bacteria belonging to internal tissue contains pathogenic microorganisms also, the normal oral microbiota. *Staphylococcus aureus* presence in both internal and external tissues for tonsils.⁴

In this study, the aim was to detect *Staphylococcus aureus* from tonsils that remove due to its recurrent also antimicrobial susceptibility for isolates and molecular identification of *mepA* gene.

Sampling

Study subjects for one year, 17 samples from outpatient with age average of 12-year-old was carried to the **Surgical Clinic** to achieve the tonsillectomy. The patient had tonsillar hyperplasia history after failures in respond to therapy of antimicrobial. Where, the tonsil was put in sterile container.

Samples Processing

The tonsils samples were homogenized in a sterile Water and then inoculated in Mannitol Salt agar as well as blood agar for isolation of bacteria. Identified of the isolates were according to methods of standard.⁴

Detection of *mepA* gene by PCR

mepA gene was detected by using the following primers:

F: 5'- ATGTTGCTGCTGCTCTGTTC-3' (718 bp)

R: 5'- TCAACTGTCAAACGATCACG 3'

Results

Tonsils were taken from 17 patients, aged 0.9–49 year, was analyzed for one year. Age mean of patients were 12.4 year, of whom 54.8% were female and 45.4% male. 15 tonsils isolates that identified as *Staphylococcus aureus* by detection of *mepA* gene (Fig. 1).

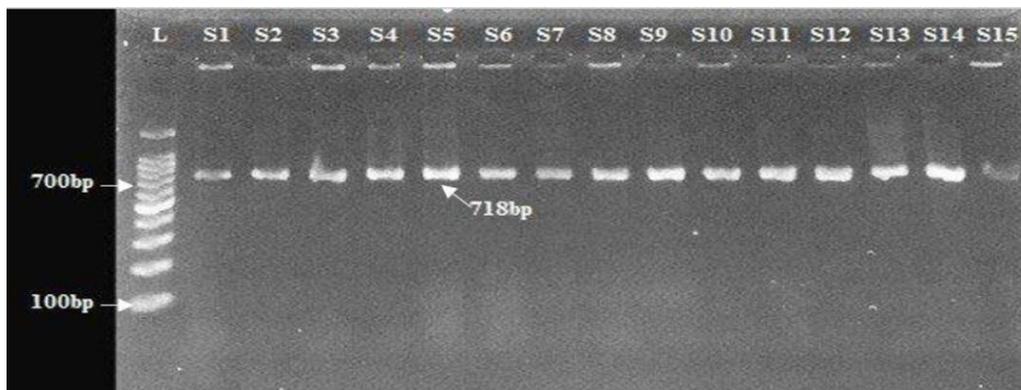


Figure 1. Gel electrophoresis for PCR amplification product of *mepA* gene (1.5% agarose, 70 voltage for 90 min), Lane L: 1500 bp DNA ladder. Lane 1-15 represent the positive results of *S.aureus* isolates (718bp)

The patients at the tonsillectomy time, had no process of acuteinflammatory.*Staphylococcus aureus* was isolated in 8 from 17 (47%) patients age0.9–37 year (mean aged = 12.8 year),in whom 77.1% had presentedhypertrophy of tonsillar, with obstruction degrees varyingbetween third and fifth degree.

In 13.0% of the patient, *Staphylococcus aureus* the only agents found, and in 17.1% threefrom *Staphylococcus aureus* with different genotypic were identified, 79.0% from17 patients, reported that before tonsillectomy was using antimicrobial.

The choice drug for treatment pharyngotonsillitis penicillin,wherethat used by 45.1% of the patients. *Staphylococcus aureus* give resistance to amoxicillin (84.7%),ciprofloxacin (27.1%), cefoxitin (25.7%) andalso amoxicillin--clavulanate (12.9%).

MostisolatesweresusceptibletoPenicillin andCiprofloxacin as show in (Table1).

Table 1. *S. aureus* susceptibility to different antibiotics

Anti-microbial	Resistance %
Penicillin	45.1
Ciprofloxacin	27.1
Cefoxitin	25.7
Erythromycin	16.4
Tetracyclin	13.1
Amoxicillin-clavulanate	12.9
Clindamycin	9.8
Ceftriaxone	4.9
Rifampin	1.6
Linezolid	1.6

Discussion

Bacterial pharyngotonsillitis that microbial infection effects on children also adolescent from (5-15) years.⁶

The mean age in this study of the patient involved was 12.3 years. Contacts of children in day care centers, home, and school, show that is cause increase in oral microbiota, leading to increase in infection recurrence.⁵ There were no notable differences in sex among people who underwent various forms of routine tonsillectomy in numerous studies were 76.6% of the patients had pharyngitis. Study show that main indications for tonsillectomy are nocturnal snoring, recurrent pharyngotonsillitis, respiratory pause, tonsillar hypertrophy and with sleep apnea.⁶

The high prevalence for *Staphylococcus aureus* in this study was (42.7%) show after inflammatory process there are bacterial persistence in the tonsils. *Staphylococcus aureus* have primary sites are throat and anterior nostril region. The main agent of tonsillitis is identification for *Staphylococcus aureus* reported by studies, with prevalence 84.1%.⁷ *Staphylococcus aureus* is presence in tonsil infections and its even after the inflammatory process that persistence in tonsillar tissue related to its ability in form biofilm, which explain therapeutic failures, therefore, infection recurrence that important in chronicity.

The isolates resistance in Ciprofloxacin and Cefoxitin was 27.1% and 25.7% to the association with penicillin.⁸ This study show resistance rate was high was due to production of lactamase enzyme. The therapeutic failures of Ciprofloxacin led to using other antimicrobials cephalosporins. *Staphylococcus aureus* that emergency for MEPA strains in the hospital environment and the community.³

In this study, two isolates found MEPA recurrent isolation in tonsillitis in Franca. In Japan, studies shown 8.9% of MEPA isolates have pharyngotonsillitis symptoms, and in other studies found that 15.9% of MEPA in patient's tonsils that submitted for tonsillectomy due to recurring tonsillitis.⁴

Therefore, no relationship between pharyngotonsillitis and MEPA. Ciprofloxacin resistance that best marker to MEPA, screening, (13.0%) from isolates that resistant to Ciprofloxacin identified as MEPA by detection of *mecA* gene. Methicillin resistance due to mutation in genes encoding lactamases overproduction or by normal PBP.⁵ These mutations generate modifications in structural, which alter proteins binding with lactams antibiotics by determining antimicrobial resistance and decreasing their affinity. PBPs overexpression occur by mutations that lead to small resistance but give significant increase in antibiotics for lactam.²⁵ Also, *Staphylococcus aureus* resistance to Cefoxitin. ⁶ *Staphylococcus aureus* producing of lactamases give resistant for penicillin enzyme. Isolates have not identified for *mecA* gene by conventional PCRs. The resistance rate was 25.7% to Ciprofloxacin is worrisome. This drug is effective against agent's cause tonsillitis, including *Staphylococcus aureus*.

Resistance to MLSb detected in 8.7% of the isolates, that mediated by the gene presence, which causes therapeutic failures and relapses. For staphylococcal infections use clindamycin that therapeutic tolerated by children, and the patients infected in allergic to penicillin. ⁸ MDR strains that important in analyzing resistance for staphylococcal aureus profiles. In this study, MDR

isolates identified, in this study the patients involved treated as outpatients. Because of the direct interaction between children and youth, this strain has a very high level of transmissibility. This is virulence factor for staphylococcal aureus that cause pneumonia. *Staphylococcus aureus* is carrying lysogenic phage which contain PVL genes. The isolates analysis that demonstrated genetic diversity between them.¹

It has been discovered that any re-creation of the colonization dynamic and the persistent genetic mutation may have existed in the tonsil. These can be seen in any animal, not only *S. aureus* denies the progression from sore throat to gingivitis. By using an antimicrobial often reduces the recurrence of pharyngitis, these two treatments aid in both detection and recovery.²

References

- 1- Riley, M. et al. (2006) *Nucleic Acids Res* 34: 1-6 (corrected supplemental data from B. Wanner)
- 2- Keck, W et al. (1990) Cloning and characterization of *mepA*, the structural gene of the penicillin-insensitive murein endopeptidase from *Escherichia coli*. *Mol. Microbiol.* 4 209-19
- 3- Baba, T et al. (2006) Construction of *Escherichia coli* K-12 inframe, single-gene knockout mutants: The Keio collection. *Mol. Syst. Biol.* 2 2006.0008
- 4- CGSC: The Coli Genetics Stock Center
- 5- Ito, M et al. (2005) Functional analysis of 1440 *Escherichia coli* genes using the combination of knock-out library and phenotype microarrays. *Metab. Eng.* 7 318-27
- 6- Kitagawa, M et al. (2005) Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *Escherichia coli* K-12 ORF archive): unique resources for biological research. *DNARes.* 12 291-9
- 7- Kohara, Y et al. (1987) The physical map of the whole *Escherichia coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. *Cell* 50 495-508
- 8- The Tn10 insertion sites determinate by Nichols et al. 1998 were reannotated by alignment with *Escherichia coli* K-12 genome sequence (GenBank accession NC-000913) P1Wu