Streptococcus pneumoniae Drugs Resistance in Acute Rhinosinusitis

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Abstract

Background: Acute rhinosinusitis that usually caused by *Streptococcus pneumoniae* becomes the reason why patients seek for medical care. Drugs resistance in *Streptococcus pneumoniae* is increasing worldwide. This study was conducted to determine drugs resistance of *Streptococcus pneumonia* from acute rhinosinusitis in Dr. Hasan Sadikin General Hospital.
Methods: A descriptive laboratory study was conducted in June–October 2014 at the Laboratory of Microbiology Faculty of Medicine Universitas Padjadjaran. The sample was taken using nasopharyngeal swabbing from 100 acute rhinosinusitis patients in Dr. Hasan Sadikin General Hospital and planted on tryptic soy agar containing 5% sheep blood and 5 µg/ml of gentamicin sulphate and then incubated in 5% CO2 incubator at 37°C for 24 hours. The identification of *Streptococcus pneumonia* was performed by optochin test. The susceptibility test against *Streptococcus pneumoniae* was done using disk diffusion method.The antibiotic disks were trimethoprim-sulfamethoxazole, oxacillin, levofloxacin, azithromycin, and doxycycline.
Results: Out of 100 samples, 8 of them were tested positive for *Streptococcus pneumoniae*. Three of *Streptococcus pneumoniae* isolates died with unknown reason after it were stored at -80 .The drugs resistance test showed the resistance of *Streptococcus pneumonia* to oxacillin, azithromycin and trimethoprim were 6, whereas levofloxacin and doxycycline are 4.
Conclusions: *Streptococcus pneumonia* drugs resistance in acute rhinosinusitis shows the resistance of *Streptococcus pneumoniae* to oxacillin, azithromycin and trimethoprim are 6, whereas the resistance to levofloxacin and doxycycline are 4. **[AMJ.2016;3(1):64–8]**

Keywords: Acute rhinosinusitis, drugs resistance, *Streptococcus pneumoniae*

Introduction

Streptococcus pneumonia (S. pneumoniae) of acute bacterial rhinosinusitis occurs in adults is 20 to 45% whereas in children is 30 to 43%.[1] According to European position paper on rhinosinusitis and nasal polyps 2012 (EPOS 12), rhinosinusitis is an inflammation on the nose and paranasal sinuses mucosa, characterized by two or more symptoms, with one symptoms should include nasal congestion, their nasal discharge, facial pain in the sinus area or reduction of smell. Acute rhinosinusitis is when the symptoms last less than 12 weeks and experiencing complete resolution.[2]

Data on the Department Otorhinolaryngology-Head and Neck Surgery Dr. Hasan Sadikin General Hospital in 2008 found comorbid allergic rhinitis patients in the form of rhinosinusitis as much as 75%. Whereas in 2010, the incidence of rhinosinusitis is about 44%.[3]

The first line therapy for acute bacterial rhinosinusitis in Department of Otorhinolaryngology-Head and Neck Surgery, Dr. Hasan Sadikin General Hospital is penicillin beta lactam combine with beta lactamase inhibitor; second line is floroquinolone and macrolide. According to Infectious Disease Society of America clinical practice guideline, the first line therapy for acute bacterial rhinosinusitis in adults is amoxicillin-clavulanate.[4] However, amoxicillin as the first-line therapy are recorded in American Family Physician.[5] For patients who allergic to

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penicillin, trimethoprim-sulfamethoxazole, a macrolide such as azithromycin, doxycycline and levofloxacin may be used as an alternative to amoxicillin.[5,6]

High medical costs and high mortality rates are associated with high *S. pneumoniae* antimicrobial resistance rates.[7,8] This study was aimed to describe the pattern of drugs resistance, *S. pneumonia* from acute rhinosinusitis in Dr. Hasan Sadikin General Hospital. It is beneficial to carry out a study on drugs resistance about *S. pneumoniae* from acute rhinosinusitis to provide information of drug sresistance *S. pneumoniae,* and the approach to the management of drugs resistance *S. pneumoniae* infections may change greatly in the next few years.

Methods

This study was conducted using secondary data descriptive laboratory method. The susceptibility test was conducted from June to October 2014 at the Laboratory of Microbiology Faculty of Medicine Universitas Padjadjaran Bandung. The study was already approved by Health Research Ethics Committee. The samples of this study were 8 *S. pneumonia* that was isolated from specimens of 100 adult patients with acute rhinosinusitis in Dr. Hasan Sadikin General Hospital that was done in a previous research from Department of Otorhinolaryngology-Head and Neck Surgery. Dr. Hasan Sadikin General Hospital Bandung.

The specimens of 100 acute rhinosinusitis patients were taken by nasopharyngeal swabbing and were transported using amines medium. After that, swab specimens were inoculated on tryptic soy agar plates containing 5% sheep blood and 5 µg/ml of gentamicin sulfate. Then, tryptic soy agar plates were incubated in 5% CO_2 incubator at 37°C for 24 to 48 hours. Colonies of *S. pneumoniae* appears as a small, grey, moist (sometimes mucoidal), colonies and characteristically produce a zone of alpha-hemolysis (green) on tryptic soy agar plates.[9]

For confirmation of *S. pneumoniae* colonies, identification test of *S. pneumoniae* such as gram staining, catalase test,optochin test and bile solubility test should be done.

After *S. Pneumoniae* isolates were identified, antimicrobial susceptibility testing of *S. pneumoniae* isolates were started by disk diffusion method. Mueller-Hinton agar medium supplemented with 5% sheep blood is recommended. The agar plates should have a uniform depth of 3 to 4 mm. The inoculum for antimicrobial susceptibility testing of *S. pneumoniae* from fresh pure cultures of *S. pneumoniae* was grown overnight on blood or chocolateagar was prepared. Cell suspensions of the bacteria were prepared to be tested in the sterile physiologicalsaline or Mueller-Hinton broth. A cell suspension equal to a density of a 0.5 McFarland turbidity standard was used for the inoculum. Viable colonies from an overnight sheep blood agar plate were suspended in a tube of broth to achieve a bacterial suspension equivalent to a 0.5 McFarland turbidity standard. This suspension should be used within 15 minutes. The density of the suspension was compared to the 0.5 McFarland turbidity standard.[9]

After the plate was dry, the antimicrobial disks such as levofloxacin, doxycycline, trimethoprim-sulfamethoxazole, azithromycin and oxacillin were placed on the plates. Sterile forceps was used to place the disks on the Mueller Hinton agar and tap them gently to ensure they adhere to the agar. The plates were incubated in an inverted position in 5% CO2 atmosphere for 20 to 24 hours at 35°C. After an overnight incubation, the diameter of each zone of inhibition was measured by a ruler or callipers. The antimicrobial susceptibility of the test strain was interpreted by comparing the results to the Clinical and Laboratory Standards (CLS) Institute standard zone sizes and European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard zone sizes.[9]

Results

Out of 100 samples, 8 of them were tested positive for *S. pneumoniae*. Three of *S. pneumoniae* isolates died with unknown reason after it was stored at -80 .

The drugs resistance test showed the resistance of *S. pneumonia* to oxacillin, azithromycin and trimethoprim were 6, whereas levofloxacin and doxycycline are 4. (Figure 1)

Discussions

S. pneumonia was chosen in this study because *S. pneumonia* is one of the main etiology in acute rhinosinusitis. From previous study, it was described that there were 54% bacterial isolates from 100 patients with acute rhinosinusitis such as 17% *Staphylococcus epidermidis*, 8% *S. pneumoniae*, 6% *Staphylococcus aureus*, 5% *Enterobacterdoacae*,

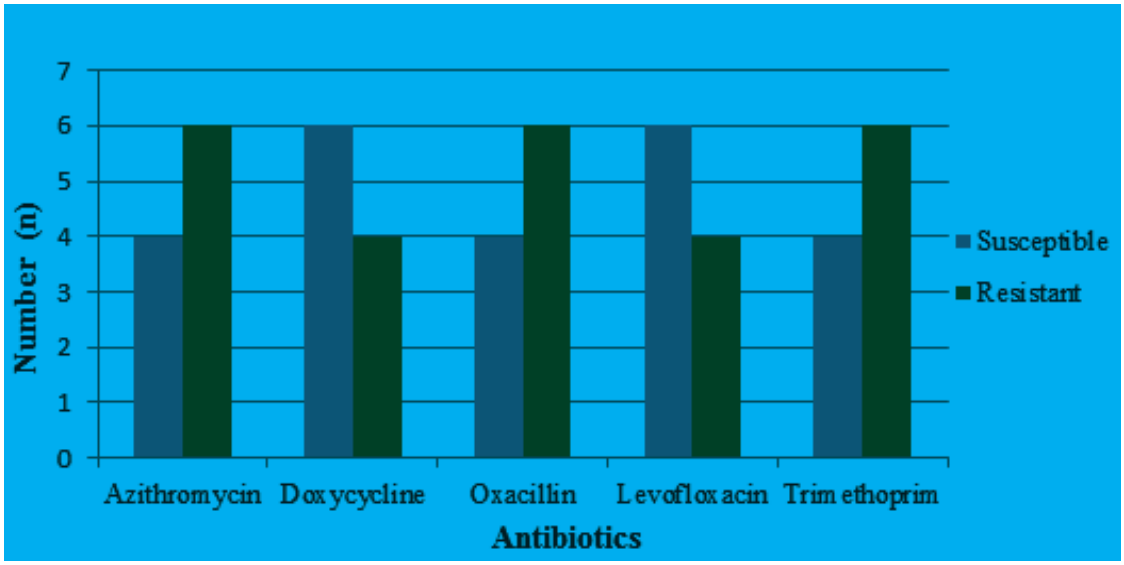

Figure 1 Antimicrobial Susceptibility Test of five S. pneumonia isolates

2 % *Streptococcus viridans*, 2% *Staphylococcus saprophytious*, 2% *Moraxella sp*, 1% *Klebsiellapneumoniae*, 1% *Haemophillus influenzae*, 2% *Pseudomonas aeruginosa*,1% other *Haemophillus,* 1% *Pseudomonas luteola* , and 1% *Serratialiquefaciens*.[3]

The sample sizes that are needed for this study are 65 *S. pneumoniae* isolates. *S. pneumonia* occurs in acute bacterial rhinosinusitis in adults is 20 to 45%.[1] In this study, 8% samples were positive for presence of *S. pneumonia*. This is because specimens of 100 acute patients with acute rhinosinusitis were taken using nasopharyngeal swabbing with Dacron swabs and the swabs were transported and stored in amies medium based on the previous research from Department of Otorhinolaryngology-Head and Neck Surgery Dr. Hasan Sadikin General Hospital Bandung. There are some evidences that rayon swabs perform better than Dacron swabs for the culture of pneumococci from nasopharyngeal swabs.[10] Besides, there is recommendation that rayon swabs are transported and stored in skim milk-tryptone-glucose-glycerin (STGG) prior to bacterial culture.[11] All specimens should be processed within 6 hours after collection.[12]

S. pneumoniae isolates survived for at least 3 years in STGG (skim milk, tryptone, glucose, glycerol) medium at -80°C.[13] However, out of 8 samples of *S. pneumoniae* isolates, there were 3 samples isolates died after it is stored at -80°C with STGG medium. Furthermore, some evidences showed the toxins produced by bacteria that kill or inducesuicide (apoptosis and autolysis) in genetically identical members of their own species such as the murein hydrolases and the choline-binding proteins responsible for autolysis and allolysis in *S. pneumoniae.*[14]

In this study resistance of *S. pneumoniae* to oxacillin is 6. Oxacillin that is an alternative to beta lactams group such as amoxicillin-clavulanate which is the first line therapy for acute bacterial rhinosinusitis in adults.[4] This condition is happen because beta lactams group do not provide reproducible results used in this study. If the zone of inhibition around the oxacillin disk is less than 20 mm which means resistant, additional minimum inhibitory concentration (MIC) testing must be performed to assess whether the isolate is resistant or susceptible to penicillin.[9] Global surveillance studies have shown that -lactam-nonsusceptibility rates of *S. pneumoniae* increased in the worldwide during 1990s and 2000s because structural changes in the penicillin targets, the penicillin-binding proteins 1a, 1b, 2x , 2a, 2b and 3.[15] In addition, increased resistance among penicillin-non-susceptibility *S. pneumoniae* is due to a mutation in penicillin binding protein 3 that cannot be overcome by the addition of a lactamase inhibitor.[4] On the other hand, similar results showed 56 % of the *S. pneumoniae* isolates were penicillin-resistant.[16] The study in US showed that the highest penicillin-non-

susceptibility *S. pneumoniae* were found in South Africa(74%),the Far East(63%) and the Middle East (54%).[15]

Resistance of S. pneumoniae to azithromycin is 6. According Chow et al.[4] and Liñares et al.[15] showed the emergence of *S. pneumoniae* that carry both *erm*B and *mef*E macrolide resistance genes is a cause for concern, especially in Asia countries, Russia, South Africa, and the USA. On the other hand, the increasing in the prevalence of macrolide resistance is strongly correlated to prior antibiotic use, particularly macrolides such as azithromycin, lactams, and trimethoprim.[4,15] Some similar studies showed the rates of resistance to azithromycin range from 22% to 67% for S. pneumoniae.[16] Besides, macrolide resistance in Europe was high in isolates collected such as France(55.6%) were also reported.[15]

In this study, the resistance rate of *S.pneumoniae* to trimethoprim is as the same as the result during oxacillin susceptibility test of *S. pneumoniae*. Indeed, reports from similar studies showed the rates of resistance to trimethoprim range from 50% to 75% for *S. pneumoniae*. The study by Chow et al.[4] showed that the resistance to trimethoprim among *S. pneumoiae* isolates occurs because of the mutations in the dihydrofolate reductase gene and prior exposure to trimethoprim, macrolides or penicillin.

In this study resistance of *S. pneumonia* to levofloxacin is 4, because the mutation point producing amino acid changes in the quinolone resistance-determining regions of the subunits of DNA topoisomerase IV and DNA gyrase.[15] However, some studies showed that resistance rate of *S. pneumoniae* to levofloxacin was 1.4% to 1.6% in more than 500 *S. pneumonia*e isolates.[17,18]

Doxycycline is active against *S. pneumoniae.* Data from national surveys in Canada showed that Doxycycline is highly active against *S. pneumoniae* (93.2%).[18] Similar reports from England, Wales, and Northern Ireland, reveal that invasive isolates of *S. pneumoniae* have remained highly susceptible to doxycycline(91%).[3] However, susceptible rate of S. pneumoniae to doxycycline is 6 because of the tetracycline resistance determinant (*tet*M) related to *erm*B macrolide resistance gene.[19]

There are several limitations in this study. First, limited *S. pneumoniae* isolates because of the lack of time in collecting the data and lack of acute rhinosinusitis patients in Dr. Hasan Sadikin General Hospital. Several steps that need to be considered for further improve the research include the use of larger sample size taken more different area which is the different hospital in Bandung to yield a better result.

There are also a few steps that need to be concerned in order to prevent the spread of drugs resistance *S. Pneumoniae* in both community setting and health-care setting, so that the further complication can be prevented. For example, reduce antibiotic use in communities and increased understanding of other factors that contribute to the development and transmission of resistance. The most important way to reduce *S. pneumoniae* infections is to increase the use of existing polysaccharide vaccines and to begin to use of new polysaccharide-protein conjugate vaccines in young children. After that, educate the community and staff in health-care facilities about covering mouth and nose while sneezing and coughing and personal hygiene such as hand washing.

As a conclusion, drugs resistance *S. Pneumoniae* from acute rhinosinusitis patient in Dr. Hasan Sadikin General Hospital showed the resistance of *S. pneumoniae* to oxacillin, azithromycin and trimethoprim are 6 whereas resistance to levofloxacin and doxycycline are 4.

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