



## ANTIMICROBIAL SUSCEPTIBILITIES OF PNEUMOCOCCAL BACTERIA ISOLATED FROM ADULTS PATIENTS IN A CERTAIN HOSPITAL IN INDONESIA

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### ABSTRACT

Respiratory tract infections are serious prevalent infectious diseases which cause increase of morbidity and mortality worldwide including in Indonesia. Antibiotics used to treat these infections give effective response, but the problem emerges concerning the increasing prevalence of antibacterial resistance because of irrational use of antibiotics. This study was aimed to identify strains of bacteria isolated from respiratory tract infectious patients of adults in one of the hospitals in West Java of Indonesia and determine susceptibility of bacteria to some antibiotics. Identification of bacteria was done based on the morphological examination and gram staining test, followed up by the 16S rRNA PCR-Sequencing method for a final confirmation of the strains of bacteria. Susceptibility of bacteria was tested by agar diffusion method, and the data were compared with the related data in reference book of Clinical Laboratory Standards Institute (CLSI). The results indicated that nine strains of bacteria were identified from ten sputum samples and most were gram negative bacteria, *Citrobacter koshleri*, *Enterobacter Sp.*, *Pseudomonas aeruginosa*, *Acinetobacter bereziniae*, *Klebsiella Varicola*, *Stenotrophomonas pavanii*, and *Acinetobacter baumannii*, and two strains were gram positive bacteria, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Most of the bacteria were resistant to amoxicillin and cefadroxil, some were resistant and intermediate to ceftriaxone and cefotaxime, some were resistant and susceptible to trimethoprim, and most were susceptible to sulfamethoxazole. This study concluded that the bacteria identified had variation in susceptibility to antibiotics tested, where most were resistant to  $\beta$ -lactam antibiotics and were susceptible and intermediate to sulfamethoxazole.

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## INTRODUCTION

Respiratory tract infections are one of the most prevalent infectious diseases. These infections consisting of upper and lower respiratory tract infections are a common cause of morbidity and mortality worldwide including in Indonesia. Approximately 40-70% of children patients visiting hospitals are those suffering from acute respiratory infections (Department of Health of Indonesia, 2002). Health Ministry of Indonesia reported 7.2 millions of cases of acute respiratory infections in 2007 and this number increased to 18.7 millions in 2011 (Ministry of Health of Indonesia, 2007).

Respiratory tract infections are generally caused by several kinds of microorganisms, but mainly due to viruses and bacteria. Bacterial pathogens have an important etiological role and some are known as the most common cause of childhood respiratory tract infections, such as *Streptococcus pneumoniae*, *S. pyogenes*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Mycoplasma pneumoniae* (Edmond *et al.*, 1996; McCracken, 2000; Gleason, 2002; Principi and Esposito, 2002). Antibiotic treatment effectively responds to the cases of respiratory infections, however, irrational uses of antibiotics were widespread especially among children in developing countries and often cause antibacterial

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resistance (Felmingham *et al.*, 2004). Furthermore, considerable variation in the prevalence of resistance occurs both between and within countries, and resistance of pathogens emerges rapidly in specific localities (Felmingham and Gruneberg, 2000; Felmingham, 2002; Adam, 2002).

These phenomena of the increasing prevalence of resistance of bacterial respiratory pathogens led to complication of selection of first-line treatment due to difficulty in determining the etiological agents of the respiratory infections (Felmingham and Gruneberg, 2000; Felmingham, 2002). Since infections vary with time and geographies, in identification of etiological bacteria of respiratory infections and evaluation of antibacterial resistance are clearly important for rational uses of antibiotics and effective management of respiratory tract infections.

This study was aimed to identify bacterial pathogens of respiratory tract infections amongst adults in a national hospital in West Java of Indonesia and determine susceptibility of identified bacteria to some antibiotics. In this national hospital, morbidity caused by respiratory tract infections or pneumococcal infections among adults is high enough.

## MATERIALS AND METHODS

### Sample collection and Processing

Samples were collected from sputum of adults with pneumococcal infections hospitalized in a national hospital in West Java of Indonesia. The samples were sent to the Laboratory of Microbiology of Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia for processing and analyses. The processing of the samples was carried out according to standard guidelines. Briefly, samples of sputum were taken, collected in a small plastic tube, and 3 ml of the specimen was put into sterile Trypticasein Soy Broth (TSB) media, then incubated at 37<sup>o</sup> C for 18-24 hours. One ose of the suspension of bacteria was taken, scratched on the solid Trypticasein Soy Agar (TSA) prepared before, and incubated at 37<sup>o</sup> C for 18-24 hours. Suspicious colonies were then subcultured in new TSA media for purification and thereafter stored in the refrigerator for subsequent analysis.

### Identification of bacteria

Preliminary identification of isolates was done based on the morphological examination and gram staining test. The identification was then followed up according to the 16S rRNA PCR-Sequencing method for a final confirmation of the isolates. Isolation of total DNA was performed following isolation protocol of GeneJET™ Genomic DNA Purification Kit®, and amplification of the 16S rRNA gene was carried out by the PCR method. The primer 1492R (5'GGTTACSTGTGTTACGAC3') and primer 27F (5'AGAGTTTGATCMTGGCTCAG3') were used for the 16S rRNA gene amplification. DNA of bacteria resulted from PCR was analyzed by electrophoresis and DNA strands formed were observed by *mini trans-iluminator UV*.

### Sequencing of 16S rRNA fragment

Sequencing of 16S rRNA fragment was done by the use of *Basic Local Alignment Search Tool* (BLAST) of nucleotide sequence which was matched with the data base.

### Bacterial susceptibility testing

Susceptibility of bacteria to antibiotics was tested using agar diffusion method. The antibiotics used were amoxicillin (10 µg), sefadroxil (30 µg), trimethoprim (5 µg), sulfamethoxazole (300 µg), ceftriaxone (30 µg), and sefotaxime (30 µg). In brief, each petri disc containing suspension of bacterial cultures was added liquid Moeller Hinton Agar (MHA) (40-50 °C). The mixture was then homogenized and allowed until solid. The density was compared with a barium chloride standard (0.5 McFarland). Next, antibiotic discs were placed on the plates of agar media, spacing each disc well to avoid overlapping of inhibition zones. The plates were incubated at 37 °C for 24 hr, and the diameters of inhibition zones were recorded. The results were compared with the related data available in reference book of Clinical Laboratory Standards Institute (CLSI) 2007.

### Place of study

This study was carried out in a national hospital in Garut regency, West Java of Indonesia.

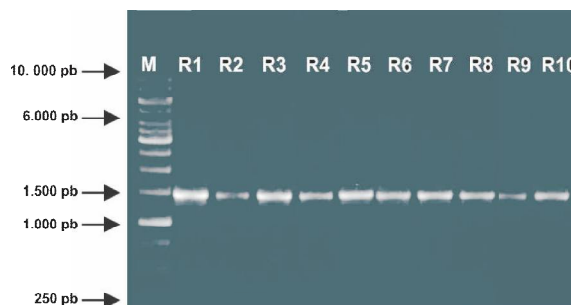
### Ethical clearance

Before this research was performed, the proposal was submitted to the Research Ethical Committee of Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia for approval of Informed Consent.

## RESULTS

### Strains of bacteria identified from samples

Ten bacteria were isolated from 10 samples of sputums derived from 10 adults with pneumococcal infections hospitalized in a certain hospital in West Java of Indonesia. The bacteria were identified based on the morphological examination and gram staining test, followed up by an application of the 16S rRNA PCR-Sequencing method. Nine strains of bacteria were identified as shown in Fig 1 and Table 1.



**Fig 1** DNA visualization resulted from amplification of genes encoding 16S rRNA

Results of DNA visualization indicated one strand of DNA parallel with the marker used at the base size of 1500 pb (Fig. 1). The strand matched with the restriction

of DNA strand resulted from the primer 1492R and primer 27F, 1500 pb (Janda and Abbott, 2007).

**Table 1** Nucleotide BLAST of DNA of 16S rRNA bacteria on samples R1-R10

Sample	Identified Bacteria	Gram of bacteria	Homology (%)
R1	<i>Citrobacter koseri</i>	Gram Negative	99
R2	<i>Enterobacter Sp.</i>	Gram Negative	100
R3	<i>Pseudomonas aeruginosa</i>	Gram Negative	100
R4	<i>Acinetobacter bereziniae</i>	Gram Negative	100
R5	<i>Klebsiella Varicola</i>	Gram Negative	99
R6	<i>Stenotrophomonas pavanii</i>	Gram Negative	100
R7	<i>Staphylococcus aureus</i>	Gram Positive	100
R8	<i>Acinetobacter baumannii</i>	Gram Negative	100
R9	<i>Staphylococcus epidermidis</i>	Gram Positive	100
R10	<i>Staphylococcus epidermidis</i>	Gram Positive	100

Table 1 shows that nine different types of bacteria were isolated and identified from 10 samples, in which each bacterium derived from each different sample, except *S. epidermidis* was identified from samples R9 and R10. Most of the bacteria identified belong to gram negative, and only two strains of gram positive bacteria were found, i.e. *S. aureus* and *S. epidermidis*.

#### Susceptibility of bacteria

Identified bacteria were tested for their susceptibility to some antibiotics i.e. amoxicillin (10 µg), sefadroxil (30 µg), trimethoprim (5 µg), sulfamethoxazole (300 µg), ceftriaxone (30 µg), and sefotaxime (30 µg). These antibiotics were commonly used in the treatment of pneumococcal infections of patients treated in the hospital where this study was carried out, with different frequency of use. Their inhibition zone diameter was compared with that reported in the standard and guideline of CLSI 2007.

**Table 2** Susceptibility of *Citrobacter koseri*, *Enterobacter Sp.*, *Pseudomonas aeruginosa*, *Acinetobacter bereziniae*, *Klebsiella Varicola*, *Stenotrophomonas pavanii*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Staphylococcus epidermidis* to amoxicillin, cefadroxil, trimethoprim, sulfamethoxazole, ceftriaxone, and cefotaxime.

Antibiotics	Zone Diameter of Inhibition (mm)								
	<i>C. koseri</i>	<i>E. Sp.</i>	<i>P. aeruginosa</i>	<i>A. bereziniae</i>	<i>K. varicola</i>	<i>S. pavanii</i>	<i>S. aureus</i>	<i>A. baumannii</i>	<i>S. epidermidis</i>
Amoxicillin	Niz (r)	Niz (r)	Niz (r)	17.26 (r)	Niz (r)	2.19 (r)	7.36 (r)	14.19 (i)	Niz (r)
Cefadroxil	Niz (r)	Niz (r)	Niz (r)	8.03 (r)	Niz (r)	15.51 (i)	12.85 (r)	11.01 (r)	6.03 (r)
Trimethoprim	Niz (r)	19.5 (s)	Niz (r)	4.76 (r)	7.5 (r)	21.02 (s)	12.21(i)	16.05 (s)	Niz (r)
Sulfamethoxazole	16.26 (i)	26.02 (s)	26.08 (s)	25.5 (s)	14.01 (i)	20.75 (s)	5.53 (r)	21.75 (s)	10.50 (r)
Ceftriaxone	16.54 (i)	3.04 (r)	Niz (r)	14.28 (i)	5.75 (r)	20.25 (i)	17.01 (i)	11.26 (r)	Niz (r)
Cefotaxime	17.53 (i)	2.54 (r)	5.02 (r)	20.00 (i)	10.27 (r)	10.27 (r)	20.51 (i)	14.86 (i)	5.50 (r)

Niz: No inhibition zone; r: resistant; i: intermediate; s: sensitive

Table 2 shows zone diameters of inhibition of six antibiotics tested and susceptibility categories of the bacteria which were already identified. Susceptibility categories were determined as resistant, intermediate or susceptible after zone diameters of the antibiotics tested were compared with those of standard references in CLSI 2007.

*C. koseri* was resistant to amoxicillin, cefadroxil, and trimethoprim as no inhibition zone was observed. This bacteria was not resistant to sulfamethoxazole, ceftriaxone, and cefotaxime, but its susceptibility decreased as the inhibition zone of those antibiotics was in the intermediate category according to CLSI 2007. *Enterobacter sp* was susceptible to trimethoprim and sulfamethoxazole, and resistant to other four antibiotics tested. *P. aeruginosa* was resistant to four antibiotics, and

was susceptible only to sulfamethoxazole. *A. bereziniae* was resistant to amoxicillin, cefadroxil, and trimethoprim as the diameter of inhibition zone belonged to resistant levels. It is susceptible to sulfamethoxazole and intermediate to ceftriaxone and cefotaxime.

*K. Varicola*, like *P. aeruginosa*, was resistant to all antibiotics, in exception to sulfamethoxazole, it showed susceptibility in intermediate category. *S. pavanii* was resistant to amoxicillin and cefotaxime, intermediate to cefadroxil and ceftriaxone, and susceptible to trimethoprim and sulfamethoxazole. *S. aureus* was resistant to three antibiotics, amoxicillin, cefadroxil, and sulfamethoxazole, and intermediate to three other antibiotics, trimethoprim, ceftriaxone and cefotaxime, whereas *A. baumannii* was resistant to cefadroxil and ceftriaxone, intermediate to amoxicillin and cefotaxime, and susceptible to trimethoprim and sulfamethoxazole. The last, *S. epidermidis* was resistant to all antibiotics tested.

## DISCUSSION

This study evaluated antibacterial susceptibility of some bacteria isolated from adults with respiratory tract infections in a certain hospital. Every hospital in Indonesia has its own conditions and standard operational procedures in performing health care services toward patients with infectious diseases. So, prevalence of antibacterial resistance may vary among hospitals as it is reported that considerable variation in the prevalence of resistance occurs both between and within countries (Felmingham and Gruneberg, 2000; Felmingham, 2002; Adam, 2002), An age of patients influences the prevalence

of resistance, in which children are more prone to undergo infection with resistant pathogens than adults (Reichler *et al.*, 1992; Reinert *et al.*, 2003).

The present study identified bacteria isolated from sputum of adults with respiratory tract infections. Most of the bacteria identified were gram negative, only two gram positive bacteria were found, *S. aureus* and *S. epidermidis*.

One of failures in treatment of infectious diseases is caused by resistance of bacteria to an antibiotic used. Bacteria can become resistant by adapting their structure or function in some way that prevents them from being fully inhibited or killed by the antibiotic. Simply, an antibiotic that is used to cure an infection may not work anymore. Consequently, infections with drug-resistant

bacteria may lead to longer and more costly hospital care, and may increase the risk of dying from the infection.

Therefore, all bacteria identified in this study were important to be evaluated for their susceptibility to antibiotics usually used for the treatment of respiratory tract infections in the hospital where this study was done. The susceptibility categories of the bacteria were confirmed by comparing the diameter of inhibition zone of the antibiotics tested with those available in the standard and guideline of CLSI 2007. There was variation in susceptibility categories, which indicated that each bacterium had different susceptibility to the antibiotics.

Based on the susceptibility tests, most of the bacteria were resistant to a class of  $\beta$ -lactam antibiotics, in particular to amoxicillin and cefadroxil. Almost all strains of bacteria were resistant to these two antibiotics, except *A. baumannii* was intermediate to amoxicillin and *S. pavanii* to cefadroxil. Some bacteria were resistant to ceftriaxone and cefotaxime, and some were intermediate, and no one was susceptible.

Amoxicillin and cephalosporin antibiotics (cefadroxil, ceftriaxone, and cefotaxime) are broad-spectrum antibiotics, effective in gram-positive and gram-negative bacterial infections. In the hospital concerned this study, ceftriaxone and cefotaxime belonging to a third-generation of cephalosporin are the first choice and the most frequently used for the treatment of respiratory tract infections, whereas amoxicillin and cefadroxil are also used but not as frequent as ceftriaxone and cefotaxime. However, according to this study, the effectivity of ceftriaxone and cefotaxime much decreased as some strains of bacteria tested were resistant and some strains were intermediate to these antibiotics.

Interestingly, trimethoprim and sulfamethoxazole belonging to rarely used antibacterial agents for respiratory tract infections showed better effectivity, especially sulfamethoxazole. This was indicated by the findings that most strains of bacteria were susceptible to sulfamethoxazole, and only two bacteria were resistant, *S. aureus* and *S. epidermidis*. Trimethoprim and sulfamethoxazole have different mechanism of action with  $\beta$ -lactam antibiotics, and these two antibacterial agents are available and usually used in a combination preparation. In this study these agents were separately investigated, because a combination preparation contains other agents as fillers that may disturb the results of the test.

The overall findings of this study present a challenge to appropriate prescribing for respiratory tract infections. However, this study was lack of number of samples investigated. The number of samples were too low to provide clear information about prevalence of bacterial resistance to antibiotics for respiratory tract infections.

## CONCLUSION

This study found that most of strains of bacteria isolated from the patients of adults with pneumococcal infections were identified as gram negative bacteria. The bacteria had variation in susceptibility to antibiotics tested, where most were resistant to  $\beta$ -lactam antibiotics, amoxicillin,

cefadroxil, ceftriaxone and cefotaxime, and susceptible and intermediate to sulfamethoxazole. Further study is required to examine susceptibility of bacteria isolated from more samples of sputum derived from pneumococcal infectious patients.

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