

Antimicrobial Susceptibility Testing for *Corynebacterium pseudodiphtheriticum*

Rudi Hendro Putranto¹, Kambang Sariadji², Khariri³

^{1,2,3}Center for Biomedical and Basic Technology of Health, National Institute of Health Research and Development Technology, Health Research and Development Body, Health Ministry of RI (National Institute of Health Research and Development, NIHRD Jakarta)

Corresponding Author: Rudi Hendro Putranto

ABSTRACT

Background: *Corynebacterium pseudodiphtheriticum* is harmless to humans and only part of commensal in the upper respiratory tracts and skin. Nevertheless over the last two decades *C.pseudodiphtheriticum* has been reported was caused disease in humans, particularly in patients with impaired immunity. Currently information about *C. pseudodiphtheriticum* resistance in Indonesia is still limited.

Objectives: This study aims to determine patterns of resistance and the effectiveness of some antibiotics against *C. pseudodiphtheriticum*.

Method: The determinations of antibiotic resistance pattern are performed by using diffusion disc agar method. using the medium Muhler hilton with the addition of 5% sheep blood. The results of antibiotic susceptibility testing for *C.pseudodiphtheriticum* showed all isolates resistance to erythromycin and tetrasikline.

Results: There are two isolates showed resistance against to penicillin. In contrary all isolates are sensitive to ciprofloxacin and ceftriaxone. Resistance profile also showed multi drug resistance against to 2, 3, 4 and 7 antibiotics such as penicillin, moxifloxacin, gentamicin, vancomycin, tetracyclines, clindamycin and linezolid.

Conclusion: There are monoresistant *C. pseudodiphtheriticum* and multi-drug resistant to some antibiotics.

Keywords: *Corynebacterium pseudodiphtheriticum*, Antimicrobial, Resistance

INTRODUCTION

Corynebacterium pseudodiphtheriticum, also known as *Corynebacterium hofmannii*, is a rod bacterium from the genus of *Corynebacterium* which has gram-positive, aerobic or facultative anaerobic characteristics. This bacterium is a commensal bacterium in humans that is often found on the skin and respiratory tract. Over the past two decades, *Corynebacterium pseudodiphtheriticum* has been reported to cause disease in humans. Especially in patients who experience immune disorders.^{1,2,3}

In general, *C. pseudodiphtheriticum* is not dangerous to humans, only commensal in the upper respiratory tract and skin. Generally can occur in someone who is immunocompromised. The death rate is quite high in industrialized countries, which is 23%. This bacterium is also considered a cause of bone and joint disease infections. In some cases these bacteria are known as opportunistic pathogens such as in patients with predisposition to end-stage renal disease, HIV infection, cirrhosis of the liver and most often are respiratory infections, urinary tract infections and infections caused by catheters. Although rarely found, this organism has been reported in cases of keratitis, conjunctivitis and endocarditis.^{4,5}

There are currently no reported data on the prevalence of cases and patterns of antibiotic resistance caused by *Corynebacterium pseudodiphtheriticum*. Case reports from some of the literature

mention an infection and cause health problems as happened at the Royal Berkshire Hospital London UK, a 54-year-old man with osteoarthritic arthralgia. In the examination of synovial fluid culture *Corynebacterium pseudodiphtheriticum* was found. Antibiotic sensitivity tests show sensitivity to methicillin, penicillin, oxacillin, vancomycin, erythromycin and gentamicin.³

Reports from Brazil suggest that from 1993 to 2006 there were 1647 strains obtained from culture results from patients treated in intensive care units (ICU). A total of 1446 strains were identified as the genus *Corynebacterium* and 113 of them were *Corynebacterium pseudodiphtheriticum* from various sample materials such as from urine, surgical wounds, peritoneal fluid, catheter tip, pneumoniae samples and incisive wounds when collecting blood samples. Sensitivity test results were also reported from 113 isolates of *C. pseudodiphtheriticum* where all sensitive to vancomycin and teicoplanin. More than 50% were resistant to oxacillin, erythromycin and clindamycin. Around 38.9% - 44.2% were resistant to penicillin and ampicillin.⁴

Another case was also reported in an immunocompromised patient in one of the patients in a hospital in Brazil who had a foot injury. Wound culture results showed *C. pseudodiphtheriticum*, while antibiotic sensitivity testing showed resistance to clindamycin and erythromycin. Other antibiotics such as gentamicin, trimethoprim-sulfamethoxazole, penicillin, vancomycin, and ciprofloxacin show sensitivity.⁶ Other cases occur in patients with pneumoniae with acute lymphocytic leukemia also reported to be related to infections caused by *C. pseudodiphtheriticum* in British Columbia Columbia hospital. Treatment is done by administering antibiotics in the form of vancomycin and erythromycin.⁷

Case of treatment of infections caused by *Corynebacterium* is currently done with the choice of antibiotics including

penicillin, cephalosporins, aminoglycosides, fusidic acid, and vancomycin. In a state of allergy to penicillin, cefotaxime treatment can be used.⁷

In laboratory examinations of cases of outbreaks of diphtheria from West Kalimantan, there were 4 isolates that were confirmed to the Bacteriology Laboratory for the Center for Biomedical and Basic Health Technology suspected of *C. diphtheriae*. The isolates were then re-identified including microscopic examination, culture, isolation and bacterial identification, toxigenic tests with polymerase chain reaction (PCR) and antimicrobial tests with several antibiotics. Confirmation of the examination showed that the 4 isolates were *C. pseudodiphtheriticum* bacteria.

At present the type of bacterial *C. pseudodiphtheriticum*, although commensal, is in some cases a serious impact on health problems. Currently there is no information on the pattern of antibiotic resistance to *C. pseudodiphtheriticum* in Indonesia. The purpose of this study was to determine the pattern of resistance and effectiveness of several antibiotics against *C. pseudodiphtheriticum*.

METHOD

The study was conducted at the Bacteriology Laboratory of the Center for Biomedical and Basic Technology in Health, Balitbangkes Ministry of Health. Samples used were 4 isolates of *C. pseudodiphtheriticum* which were stored as a result of an investigation of diphtheria outbreaks between 2010 - 2015. The isolate samples were regenerated and identified conventionally using Cystine Tellurite Blood Agar (CTBA) Blood Agar (BA) and API Coryne products. Toxicity testing by looking for the presence of a tox gene was carried out using the PCR method.⁸ The isolate was then purified using Blood Agar. Determination of resistance using the disk method for diffusion using the Muhler hilton agar medium with sheep blood 5%.^{5,9,10} There are 12 disc antibiotics of

oxid products consisting of erythromycin (15 µg), rifampicin (5 µg), linezolid (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), gentamicin (10 µg), Trimethoprim sulfamethoxazole. (15 µg) vancomycin (30 µg), tetracycline (30 µg), ceftriaxone (30 µg), ceftriaxone (30 µg) and ceftriaxone (30 µg) benzyl penicillin (10 µg). Due to the absence of standard disk diffusion method breakpoints from the Clinical and Laboratory Standards Institute (CLSI) for *C. pseudodiphtheriticum*

bacteria, the break point values of streptococcus spp. was used, while for penicillin a standard breakpoint of Staphylococcus aureus was used.6 using Streptococcus pneumoniae control ATCC 49619.^{9,10}

RESULTS

The results of the sensitivity test of 4 isolates of *C. pseudodiphtheriticum* against several antibiotics using the disc diffusion method can be seen in table.1.

Table 1. C.pseudodiphtheriticum resistance pattern against several antibiotics

No	Antibiotics	Code	Antibiotic Class	Antibiotic Sub-Class	N Sample	%R	%I	%S
1	Penicillin	PEN 10ug	Penicillin	Penicillin	4	50	0	50
2	Ciprofloxacin	CIP 5 ug	Quinolone	Fluoroquinolone	4	0	0	100
3	Moxifloxacin	MFX 5 ug	Quinolone	Fluoroquinolone	4	0	25	75
4	Gentamicin	GEN 10 ug	Aminoglycoside		4	0	50	50
5	Vancomycin	VAN 30 ug	Glycopeptide	Glycopeptide	4	50	25	25
6	Tetracycline	TCY 30 ug	Tetracycline		4	100	0	0
7	Clindamycin	CLI 2 ug	Lincosamide		4	75	0	25
8	Linezolid	LNZ 30 ug	Oxazolidine		4	25	0	75
9	Rifampicin	RIF 5 ug	Ansamycin		4	50	0	50
10	Trimethoprim sulfamethoxazole	SXT 25/ 23,75 ug	Folate pathway inhibitors		4	75	25	0
11	Entromycin	ERY 15 ug	Macrolide		4	100	0	0
12	Ceftriaxone	CRO 30 ug	Cephems	Cephalosporin III	4	0	0	100

Note: %R: Percent of resistance, %I: Percent of Intermediate, %S: Percent of sensitivity

Table 2 shows *C. Pseudodiphtheriticum* strains with resistance profiles of two or even several antibiotics as multidrug resistant (MDR). These antibiotics include tetracycline, clindamycin, vancomycin penicillin, gentamicin, moxifloxacin and linezolid.

Table.2. Resistance profile of *C. pseudodiphtheriticum* against several antibiotics

Profile of resistance	N. Isolate	% Isolate.
TCY, CLI	1	25
PEN, VAN, TCY	1	25
GEN, VAN, TCY, CLI	1	25
PEN, MFX, GEN, VAN, TCY, CLI, LNZ	1	25
Total	4	100

TCY: tetracycline, CLI: clindamycin, VAN: vancomycin PEN: penicillin, GEN: gentamicin, LNZ: linezolid, MFX: moxifloxacin

DISCUSSION

C. pseudodiphtheriticum detected in samples of extraordinary diphtheria events may be of normal flora. In some cases immunocompromise can cause infection and may be responsible for airway infections such as pneumoniae and bronchitis. In hospitalized patients, sometimes *C.*

pseudodiphtheriticum can cause infectious complications in endotracheal intubation related to lower respiratory tract infections. Administration of adequate antibiotics can eliminate bacterial colonization.

In the antibiotic sensitivity test all *C.pseudodiphtheriticum* isolates showed resistance to erythromycin and tetracycline. There are 2 isolates showing resistance to other antibiotics such as penicillin. In the sensitivity test of 113 *C. pseudodiphtheriticum* isolates in 1993 - 2006 in Brazil showed almost >50% resistant to erythromycin and 47% against tetracycline and 38.9% against penicillin.4 Report of sensitivity test for *C. pseudodiphtheriticum* from samples originating from the airways in Turkey shows *C. pseudodiphtheriticum* is resistant to penicillin 66%, erithromycin 33% and all are resistant to clindamycin.¹¹

The incidence of *C.pseudodiphtheriticum* resistant to erythromycin is probably due to the frequent

exposure of these bacteria to low-dose erythromycin antibiotics given that under normal conditions *C.pseudodiphtheriticum* is a normal flora in the airways. The erythromycin resistant *C.pseudodiphtheriticum* isolate involves the ErmX gene that expresses the methyltransferase enzyme. In erythromycin resistance which is a macrolide group is thought to occur due to exposure to low-dose erythromycin which causes expression of the enzyme methyltransferase (ErmX). ErmX causes methylation at 23S rRNA at point N-6 of adenosine 2058 (A2058) which is an important nucleotide in the binding process of the macrolide-linkosamide-streptogramin B (MLS_B) antibiotic group. As a result of this methylation process, the erythromycin bond in the target is disturbed. Resistance to tetracycline is caused by the production of different cytoplasmic membranes and prevents binding of tetracycline in the ribosomal 30s subunit, so that protein synthesis can continue. Another resistance mechanism is through an efflux pump, based on the tetracycline transport out cells rapidly, thereby preventing the accumulation of tetracycline at toxic doses, so that bacterial protein synthesis is not inhibited. Resistance to penicillin can also arise due to bacteria having a limited outer membrane system, which prevents penicillin from reaching the cytoplasmic membrane (the location of penicillin binding protein). Vancomycin resistance develops due to the presence of enzymes in resistant bacterial cells, which will remove alanine residues from the peptide peptidoglycan portion.^{12,13}

Some researches also reported that *C.pseudodiphtheriticum* isolates were generally sensitive to β -lactam, aminoglycoside group, rifampicin and tetracycline.¹¹ On the contrary, the results of tests conducted in this study of 4 *C.pseudodiphtheriticum* isolates showed that only ciprofloxacin and ceftriaxone were sensitive.

Furthermore, each isolate showed the presence of Multidrug Resistance (MDR), a condition where bacteria are

resistant to at least two or more types of antibiotics. The existence of this MDR can be caused by several factors, among others is the use of antibiotics that do not meet the predetermined rules, namely the right dose, the right diagnostic and the exact bacterial cause.^{13,14,15} From the four isolates of *C. pseudodiphtheriticum* there was one isolate that was resistant to 2 antibiotics namely tetracycline and clindamycin, then there was one isolate that was resistant to 3 antibiotics namely penicillin, vancomycin and tetracycline. One other isolate showed resistance to 4 antibiotics gentamicin, vancomycin, tetracycline and clindamycin, while another isolate showed resistance to 7 antibiotics penicillin, moxifloxacin, gentamicin, vancomycin, tetracycline, clindamycin and linezolid. The presence of MDR in this isolate will have an impact on treatment issues, especially in patients who experience immunity disorders and get *C.pseudodiphtheriticum* infection. In another study reported that species of *Corynebacterium* associated with opportunistic diseases showed very high antibiotic resistance significantly compared with potentially pathogenic *Corynebacterium* such as toxigenic and non-toxicogenic *C. diphtheriae*.^{16,17}

CONCLUSIONS

All *C. pseudodiphtheriticum* isolates were resistant to erythromycin and tetracycline. All isolates were also sensitive to ciprofloxacin and ceftriaxone. The resistance profile also shows the presence of MDR against 2, 3, 4 and 7 antibiotics including penicillin, moxifloxacin, gentamicin, vancomycin, tetracycline, clindamycin and linezolid.

Suggestions

Caution for opportunistic infections from *C. pseudodiphtheriticum* which shows very high antibiotic resistance is very important. Continued sensitivity testing of some antibiotics in the form of surveillance to determine the effectiveness of antibiotics

need to be performed continuously in the future.

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