World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

IDENTIFICATION OF BACTERIA FROM CLINICAL ISOLATES OF ACUTE RESPIRATORY INFECTION (ARI) PATIENTS IN CASES OF TETRACYCLINE ANTIBIOTIC RESISTANCE

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Article Received on 15/05/2019 **Article Revised on 05/06/2019** **Article Accepted on 26/06/2019**

ABSTRACT

Objective. Tetracycline is an antibiotic that is usually used for acute respiratory infections. Previous anti-antibiotic resistance studies showed that the use of tetracycline antibiotics in ARIs cases at the Tasikmalaya treatment center had experienced resistance above 50%. This study aims to identify bacteria that cause resistance to tetracycline antibiotics. **Methods.** The identification of bacteria was carried out through the method of determining the morphology of isolates, gram staining, biochemical testing. **Results.** The results of identification of bacteria in clinical isolates of patients showed that the bacteria genus Haemophilus, Staphylococcus, Corynebacterium, Streptococcus, and Bordetella were resistant to tetracycline antibiotics.

KEYWORDS: Identification of bacteria, clinical isolates, tetracycline, antibiotic resistance.

INTRODUCTION

Acute respiratory infection (ARIs) is an acute infection of any part of the respiratory tract and associated structures including sinus paranasal, middle ear and pleural cavity.[1] While the acute sense is an infection that lasts up to 14 days. ARIs is a major cause of infectious morbidity and mortality in the world. Nearly four million people worldwide die from ARIs each year. The mortality rate is very high in infants, children, and the elderly, especially in countries with low and medium income per capita.[2]

ARIs can be caused by bacteria, viruses, and rickets. Bacteria that cause ARI are Streptococcus haemolyticus, Staphylococcus, Pneumococcus, Haemophilus influenzae, Bordetella pertussis, Corynebacterium diphtheriae.[3]

Profile of resistance of clinical isolates of patients can be used as a basis for consideration to determine the main factors increasing the prevalence of ARI, especially in the city of Tasikmalaya. This study aims to identify genus of bacteria that have been resistant to tetracycline antibiotics. The results of this study are expected to complete the profile in the case of tetracycline antibiotic resistance.

MATERIALS AND METHODS

Materials

The bacterial growth media used was Mueller Hinton Agar (MHA) (Oxoid) with a concentration of 38 g / L.

The materials used for biochemical tests are agar media (Merck), NaCl (Baxter), Tryptose (Bacto) which are dissolved in distilled water, glucose (Merck), lactose (Merck), mannose (Merck), maltosa (Merck), saccharose (Merck), peptone (Oxoid), phenol red (Taylor) dissolved in distilled water, Kovac's (Bio-Rad) reagent, TSIA, methyl red reagent (HiMedia), α-naphthol (Merck) solution, KOH 40% (Merck), citrate (Equate), and hydrogen peroxide (Swan).

Methods

A. Determination of Morphology of Clinical Isolates

Determination of morphology of isolates was carried out by observing the color, colony structure and morphology that differed from each bacterium.[4]

B. Gram Staining

Gram staining is done by making spreads from each bacterial isolate on the glass object. The glass object is then inundated with gentian violet, lugol, and fuxine solution with time intervals. Then the emersion oil drops and is observed under a microscope.[5]

C. Biochemical Testing

The biochemical tests carried out included motility test, carbohydrate fermentation test, indole test, TSIA test, urease test, Methyl Red test, Voges Proskauer test and citrate test.

1) Motility test

Motility tests are performed to see the movement of the test bacteria. Test bacteria were taken using Ose and inoculated by stabbing on motile test media in the form of semi-solid. The test media were then incubated for 18-24 hours at 37°C.[7]

2) Test carbohydrate fermentation

The carbohydrate fermentation test consisted of glucose, lactose, mannose, maltose and saccharose. This test is intended to see the ability of test bacteria to degrade or break down sugar compounds and produce organic acids from each sugarEach tube was added with phenol red and durham tube in reverse position. The test media were then incubated for 18-24 hours at 37°C.[7]

3) Indole test

The indole test was carried out to look at the production of indole from the amino acid tryptophane through the enzyme trytophanase which was shown by the formation of a red ring on the surface of the culture after the Kovac's reagent was added. The test bacteria was taken by Ose and inoculated on indole test media. The test media were then incubated for 18-24 hours at 37°C and then added Kovac's reagents.[7]

4) TSIA Test (Triple Sugar Iron Agar)

The TSIA test is carried out to distinguish bacteria based on the ability to break down glucose, lactose and sucrose, and the ability to produce H2S gas which is marked by the color change of the test media to black. The test bacteria taken with Ose was then scratched into a filled tube to tilt the TSIA zigzagically. The test media were then incubated for 18-24 hours at 37°C.[7]

5) Urea Test

Urea test was carried out to see the ability of bacteria to break urea by the urease enzyme by producing pink to red after adding phenol red as a pH indicator. The test bacteria was taken with Ose then inoculated on the urease test medium. The test media were then incubated for 18-24 hours at 37°C.[7]

6) Methyl Red Test (MR)

The MR test was carried out to see the presence of mixed acid fermentation by producing red after adding red methyl reagent. Test bacteria were taken with Ose then inoculated on MR test media. The test media were then incubated for 18-24 hours at 37 ° C.[7]

7) Vogers-Proskauer Test (VP)

The VP test was performed to detect acetoin in bacterial liquid cultures which was marked with pink to bright red on culture after dropping 40% α-naphthol and KOH solution (3: 1). The test bacteria was taken by Ose and inoculated on VP test media. The test media were then incubated for 18-24 hours at 37 C and dosed with 40% α-naphthol and KOH solutions (3: 1).[7]

8) Test Simmons Citrat (SC)

The SC test was conducted to detect the ability of test bacteria to utilize citrate as a source of carbon and energy which is characterized by blue colony. The test bacteria taken with Ose were then scratched into a tube containing the media to tilt the SC zigzagically, dripping a solution of α-naphthol and KOH 40% (3: 1).[7]

RESULTS

A. Bacterial Morphology Test Results.

A total of 42 clinical isolates were obtained in resistance studies, grouped into 5 groups based on the morphology of the colonies. The morphological test results from 5 groups of clinical isolates can be seen in table 1 and figure 1.

Table 1: Morphological Test Results of Clinical Isolates.

Bacterial Group	Bacterial Morphology			
	Form	Color	Texture	Edge Shape
1	Round	Yellowish White	Convex	Jagged
2	Round	Yellow	Flat	Jagged
3	Coil	White	Convex	Irregular
4	Round	Orange	Flat	Regular
5	Dots	Yellow	flat	Irregular.

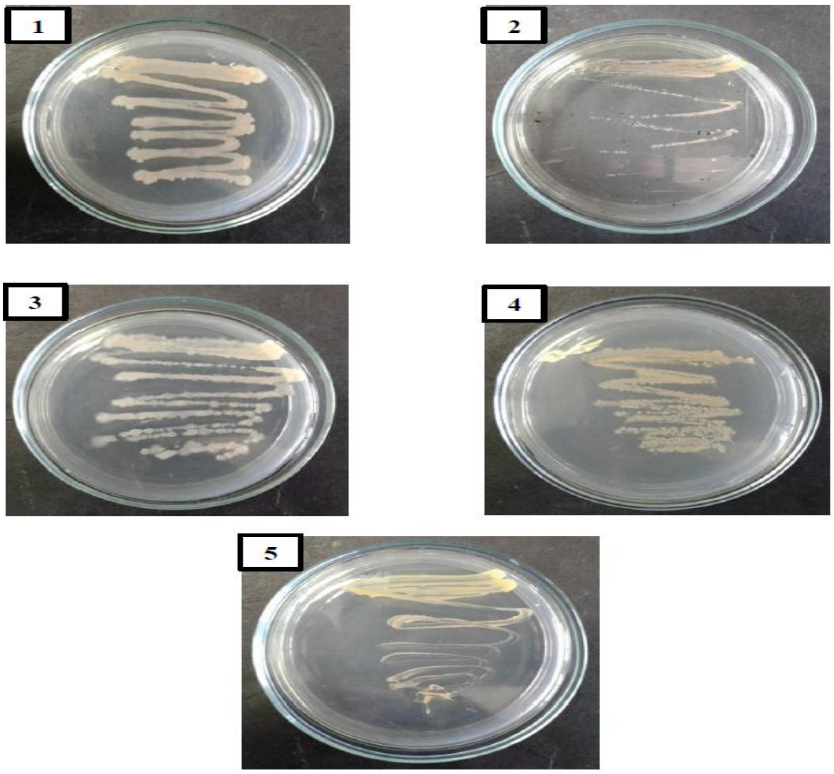

Figure 2. Morphological Test Results of clinical isolates.

B. Gram Staining Test Results.

Gram staining is intended to find out whether the bacteria belongs to the Gram-positive or Gram-negative bacteria, and to see the microscopic form of bacterial colonies. In Gram staining, four reagents are used, namely CGV (Carbol Gentian Violet) as the main dyestuff, lugol which serves to reinforce the color of the main dyestuff, alcohol which functions to remove the remaining dyestuffs, and fuxine water as a rival dye. Gram positive bacteria will retain the CGV dye so that it will appear purple or blue under a microscope. While the Gram negative bacteria will lose CGV dyes after being washed with alcohol and when given the dye the match will appear red or pink.[5]

This color difference is caused by differences in the chemical structure of the cell wall. The cell wall of Gram-positive bacteria is generally thicker than the cell wall of Gram-negative bacteria so that Gram-positive bacteria will be stronger to hold the main dye (violet). Thus, when the counter dye is given, Gram positive bacteria will not be stained.[9] The results of Gram staining on the five groups of clinical isolates can be seen in Table 2 and figure 2.

Table 2. Gram Staining Test Results.

Bacterial Group	Form	Gram
1	Cocobacillus	Negative
2	Coccus	Positive
3	Cocobacillus	Positive
4	Coccus	Positive
5	Cocobacillus	Negative

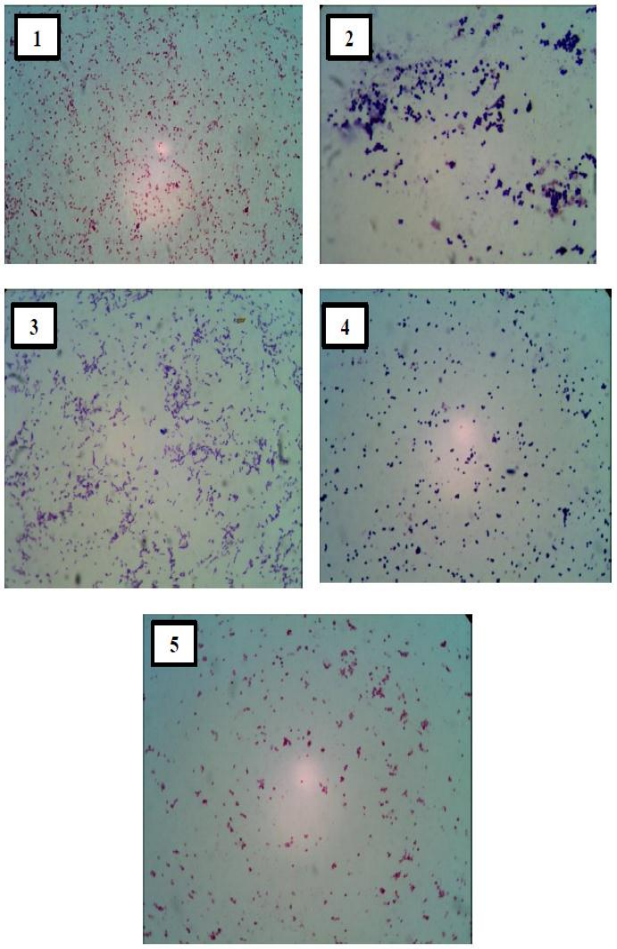

Figure 2. The results of Gram staining of clinical isolates.

C. Biochemical Test Results

Physiological or biochemical properties are important in the identification of unknown bacteria because morphologically different cultures or bacterial cells can look similar, without adequate physiological observations regarding the organic content being examined, the determination of the genus is difficult.[7]

Table 3. Biochemical Test Results for Clinical Isolates.

Biochemical Test	Bacterial Group				
	1	2	3	4	5
Glucosa	+	+	-	-	-
Lactose	+	+	-	-	+
Maltosa	+	+	+	+	-
Sakarosa	+	+	-	+	-
MR	+	+	-	+	-
VP	+	-	-	-	-
Indol	-	-	-	-	-
Motility	-	-	-	-	-
TSIA	+	+	+	+	-
SC	+	+	-	-	-
Urea	+	+	+	-	-

Based on data from a series of identification tests conducted on the five groups of clinical isolates, it can be concluded that the phenotype of the five groups of isolates is isolate 1 (Genus Haemophilus), isolate 2 (Genus Staphylococcus), isolate 3 (Corynebacterium), isolate 4 (Genus Streptococcus) and isolate 5 (Genus Bordetella). The five genus of bacteria are the bacteria that causes ARIs.[10]

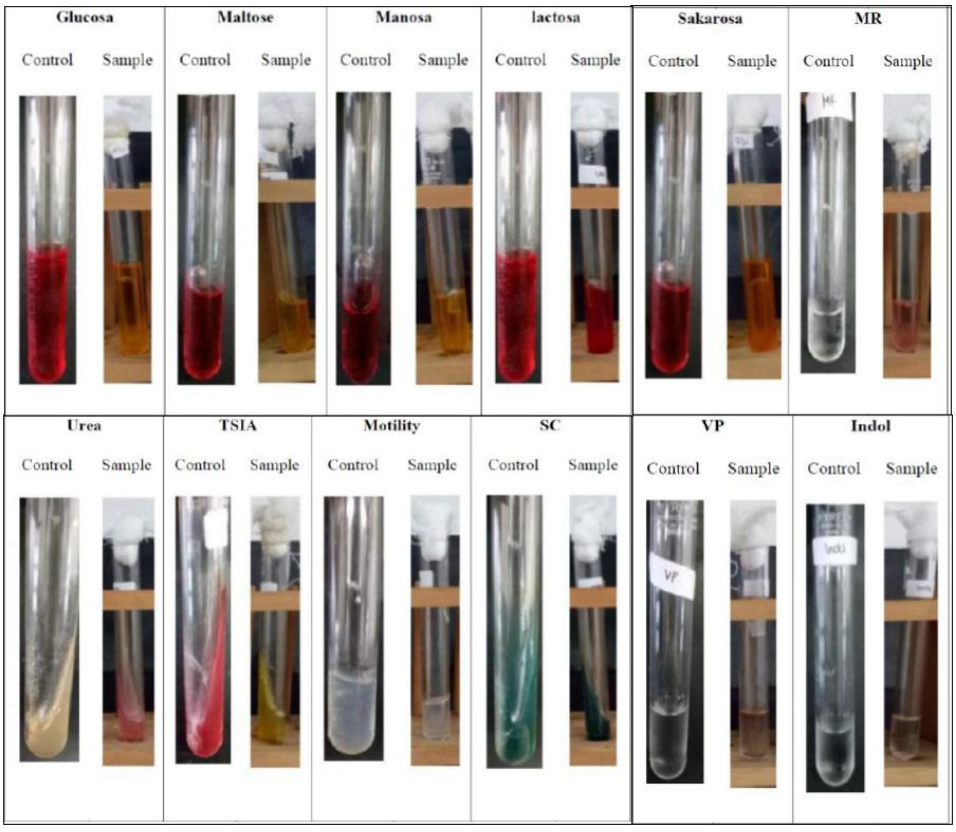

Figure 3. Biochemical Test of Clinical Isolates.

CONCLUTION

The results of identification of bacteria in clinical isolates of patients showed that the bacteria genus Haemophilus, Staphylococcus, Corynebacterium, Streptococcus, and Bordetella were resistant to tetracycline antibiotics.

ACKNOWLEDGMENTS

The author would like to thank Dede Sediana from Tasikmalaya City Health Office, also thanked Ika Khumairoh for its cooperation in this study.

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