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Original Article

PROFILE OF ANTIBIOTIC RESISTANCE AGAINST INFLUENZA IN ADULT PATIENTS: A CASE STUDY AT CITY HEALTH CENTER IN INDONESIA

DANNI RAMDHANI[1], SRI AGUNG FITRI KUSUMA[2], SHINTA NUR AZIZAH[2], IKA KHUMAIROH[2], HILARIUS BAP[2], DEDE SEDIANA[3]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia, [2]Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia, [3]Tasikmalaya City Health Office, Pharmaceutical Division, West Java, Indonesia
***Email: d.ramdhani@unpad.ac.id**

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ABSTRACT

Objective: In developing countries, cases of *Haemophilus influenzae* (HI) resistance to levofloxacin, cefixime, and tetracycline have become a serious problem in clinical treatment. This study was conducted to determine the antibiotic resistance profile of HI from adult patient isolates and to provide guidelines for more effective clinical treatment in Indonesia.

Methods: The patient isolate stock was rejuvenated, cultured on growth media and the *Kirby-Bauer* disc diffusion method was used to test for antibiotic susceptibility. Evaluation was guided by recommendations from the Clinical and Laboratory Standard Institute (CLSI).

Results: A total of 643 isolates obtained from the respiratory tract isolated and identified 73 HI strains**.** The resistance rates of the HI isolates to tetracycline, cefixime, and levofloxacin were 10.54 %, 4.31%, and 5.67%.

Conclusion: Cefixime showed more effective activity than levofloxacin and tetracycline to treat the HI strain.

Keywords: *Haemophilus influenzae*, Levofloxacin, Cefixime, Tetracycline, Resistance, Clinical isolates

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INTRODUCTION

One of the most prevalent organisms that causes community-acquired pneumonia and otitis media is *Haemophilus influenzae* (HI). Invasive pediatric illnesses, including meningitis and sepsis are also brought on by HI. These illnesses linked to HI pose a major threat to respiratory infections [1-3]. The principal method of colonization is adhering to the mucous membranes of the upper respiratory tract where it obstructs ciliary action. As a result, the respiratory system is the primary method of transmission for this species [4]. Bacteria can be classified into encapsulated and noncapsulated/nontypeable (NTHi) strains based on a polysaccharide capsule antigen. Additionally, six antigenic serotypes can be used to classify the encapsulated isolates (a-f) [5, 6]. Nearly all of the serious illnesses caused by HI type b (Hib) in children under the age of five. The World Health Organization (WHO) estimated in 2012 that Hib causes 199 000 fatalities annually and accounts for 2% of all causes of child mortality [7, 8].

Amoxicillin, cephalosporin, azithromycin, doxycycline, and fluoroquinolone are significant antibiotics used in the treatment of less severe HI infections. However, broad-spectrum cephalosporins and carbapenems are used to treat serious infections [4, 9]. Rifampin is also the medicine of choice for children with Hib who need antibiotic prophylaxis [9]. However, the WHO recently added ampicillin-resistant HI strains to the list of antibiotic-resistant bacteria and medium priority category, along with penicillin-resistant *Streptococcus pneumoniae* and fluoroquinolone-resistant *Shigella* strains, in terms of the urgent need to develop new antibiotics [10]. As a result, it is crucial to evaluate antimicrobial susceptibility trends and track the HI resistance trend in order to inform local antibiotic prescription and prioritization decisions and lower the likelihood of treatment failure.

The advent of MDR HI has sparked significant alarm among researchers, medical professionals, and government health authorities worldwide. The risk factors, effective management controls, prevention, and underlying mechanisms of acquired MDR activity have all been extensively investigated [11–13]. Despite mounting evidence that MDR HI strains have been identified in many regions. Cases of resistance to the use of tetracycline antibiotics, cefixime, and levofloxacin against HI strains isolated patients have been reported in several countries [14-16].

This study was performed to determine the antibiotic resistance profiles of HI isolates from adult patients in the city health center in Indonesia during 2017.

MATERIALS AND METHODS

The test sample consisted of a composite stock isolate from previous ARTIs (Acute Respiratory Tract Infections) research, which included 643 specific bacterial strains. The sample was purified and rejuvenated to obtain a single bacterial isolate. In accordance with the recommendations from the CLSI, Mueller Hinton Agar (MHA) (Oxoid) was employed as the bacterial growth medium and had a concentration of 38 g/l [14].

There are several biochemical test substances for identifying bacteria, including lactose (Merck), mannose (Merck), maltose (Merck), peptone (Oxoid), phenol red (Taylor), Kovac's reagent (Bio-Rad), TSIA, methyl red (HiMEdia), and α-naphthosl (Merck).

Clinical isolates are rejuvenated and purified

The process of renewing clinical isolates was done utilizing the scratch plate technique. Rejuvenated clinical isolates from earlier research were cultured at 37 °C for 18 h on fresh MHA growth medium. Different haemolytic and morphological observations of colony morphology, including colony structure, color, and other factors, were made [15].

Identification, biochemical and morphological characterization

Cellular morphology and bacterial classification were determined using the gram stain technique and microscopic observation at a resolution of x100. The isolated bacteria were classified using a conventional biochemical test after the phenotypic and cell colonies had been identified, in accordance with the biochemical testing technique currently in use [18]. GS for Gram Staining, MT for Motility Test, MR for Methyl Red, SC for Simone Citrate, TSIA for Triple Sugar Iron Agar, OX for Oxidase, UR for Urease Test, XY for

Xylose, CT for Catalase, VP for Voges-Proskauer, carbohydrate fermentation test (LAC=Lactose, MAN=Manose; MAL=Maltose; SAC=Saccharose); and IND for Indole Test are the standard biochemical tests.

Antimicrobial susceptibility testing (AST)

The sensitivity of drugs to bacterial isolates was evaluated using the *Kirby-Bauer* disc difusion test [17]. By evaluating the diameter of the inhibition zone, this test can assess the efficacy of antibiotics that are either already resistant or are still sensitive. This testing method is based on the difusion concept using antibiotic paper disks. Three times were tested during this experiment. By comparing the diameter of the inhibition zone with the standard diameter of the resistance zone created [14], it was possible to determine how resistant the tested bacteria were to cefixime and tetracycline. Table 1 shows the value for the resistance level category.

Table 1: Categories of antibiotics inhibition zone diameter based on CLSI

Categories	Tetracycline (mm)	Cefixime (mm)	Levofloxacin (mm)
Resistant	≤11	≤17	≤18
Intermediates	12-14	18-20	19-27
Sensitives	≥15	≥21	≥28

RESULTS

Biochemical conventional characterization

643 bacteria were successfully isolated and identified from patient samples who had been diagnosed with acute respiratory tract infections. Bacterial isolates were collected from patients whose cases of respiratory tract infections had been verified by a clinician. According to statistical analysis, the major bacterial genera that made up the isolates were Staphylococcus (35.5%), Streptococcus (28.5%), Bordetella (15%), Hamophyllus (11.5%), and Corynobacterium (9.5%).

Using biochemical characterization, 643 stocks of bacterial isolates classified as Gram-negative, Gram-positive, bacilli (rod-shaped), coccoid (rod-shaped), and coccobacilli were identified. Results of the conventional biochemical tests on bacteria indicated five different bacterial genus: Bordetella, Corynebacterium, Staphylococcus, Streptococcus, and Haemophilus (table 2, fig. 1).

Table 2: Results of biochemical and morphology cell identification

Grouping	Structure	Gram	Genus	Isolates	Biochemical test
1	Round-shaped	Positive	Staphylococcus	228	CT, MR, VP, UR, SC, LAC, MAN, MAL
2	Coccus-capsulated	Positive	Streptococcus	182	LAC, MAL
3	Coccobacillus-capsulated	Negative	Bordetella	95	OX, CT,UR
4	Coccobacilli	Negative	Haemophilus	77	CT, OX, MAL
5	Bacilli-rod shaped	Positive	Corynebacterium	61	MR, CT, TSIA, LAC, MAN, MAL

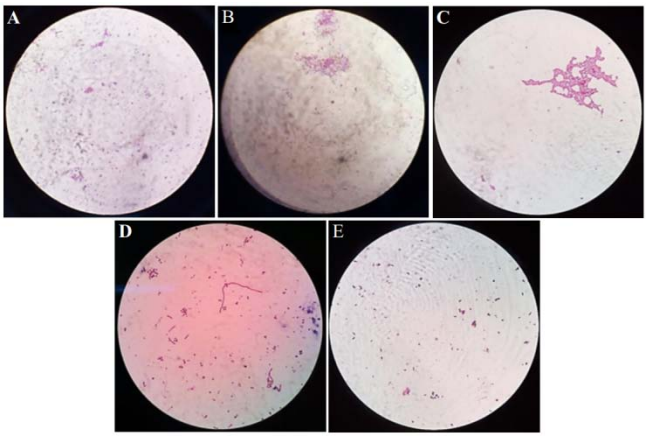

Fig. 1: Gram stain of the bacterial group: (A). Staphylococcus, (B). Streptococcus, (C). Bordetella, (D). Haemophilus, (E). Corynebacterium

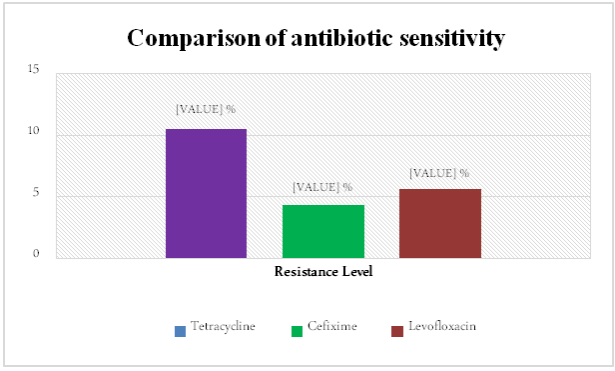

Fig. 2: Test of antibiotics resistance against *Haemophilus influenzae*

Antimicrobial susceptibility testing (AST)

The primary objective of our study was to determine the incidence of HI bacterial resistance to tetracycline, cefixime, and levofloxacin. The samples were taken from patients who were reported to have respiratory tract illnesses at a medical facility in the urban area of Tasikmalaya, Indonesia.

Our research examined at the degree of antibiotic resistance against bacterial isolates that were positively identified as HI bacteria. The test findings can be used as a guide in selecting the most effective medications to treat infections caused on by HI bacteria. Tetracycline 2–8 µg/ml, cefixime 0.5-2 µg/ml, and levofloxacin 10 µg/ml were the antibiotic disc concentrations utilized in accordance with CLSI recommendations. The susceptibility of categories can be grouped into resistant and non-resistant. The Comparison of Antibiotic Sensitivity is shown in fig. 2.

DISCUSSION

643 bacterial isolates were identified based on biochemical tests and 77 bacterial isolates showed positive results for HI. The oxidase test, catalase test, and carbohydrate (maltose) fermentation assays are biochemical procedures to precisely detect HI. A test for cytochrome oxidase can be used to identify its presence, whereby organisms with cytochrome C as part of their respiratory chain can convert the Kovac oxidase substrate, tetramethyl-p-phenylenediamine dihydrochloride, to a purple molecule [4]. In order to identify HI, this test is important. Catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide, is shown to be present in this test (H_2O_2). It is employed to distinguish between bacteria that generate the catalase enzyme. This test makes HI quickly identifiable. A popular technique for identifying microorganisms is based on their capacity to synthesize organic compounds through the metabolism of specific carbohydrates and related substances. Depending on an organism's capacity to ferment carbohydrates added to the basal medium, different fermentation media are employed to separate the organisms. Maltose fermentation was successful with HI. Performing this test is simple, and the findings are obvious.

The widespread use of major respiratory infections that exhibit high rates of antimicrobial resistance has raised substantial questions about the selection of an effective antimicrobial drug for the combination therapy of RTIs. Amoxicillin-clavulanate and oral cephalosporins have been utilized often in Indonesian private-sector hospitals for the management of outpatients with RTIs due to the high prevalence of beta-lactamase in HI.

The study of HI resistance to tetracycline antibiotics showed a relatively low resistance value of 10.5%, when compared to the results of the study in Lebanon (46.7%). The results of the evaluation of HI resistance to cefixime were also relatively low at 4.3%, when compared to the research reported in Iran at 42%. Furthermore, a study in Taiwan regarding the antibiotic resistance of levofloxacin against HI bacteria isolated from the respiratory tract showed a result of 9.2%, which was still higher when compared to the present study, which was 5.6% [8, 17, 18]. This means that the use of tetracycline, cefixime, and levofloxacin for the treatment of HI infection is still reliable. Research concerned with HI resistance in Indonesia is very rare; therefore, this information is important because it can be a guide for the selection of appropriate antibiotics in treating HI infections. The level of AB resistance obtained can provide a reference for health practitioners, especially doctors in prescribing antibiotics. Synergistic coordination between the city health office and health practitioners can be improved in continuously improving patient compliance in taking antibiotics.

In this study, the highest resistance was demonstrated by tetracycline, compared to cefixime and levofloxacin. The resistance mechanism can cause the bacterial cell to be destroyed by adhering to the 30S subunit of the bacterial ribosome and blocking tRNA from binding to either the A or P sites [19, 20]. The tet(B) gene, which is typically found on conjugative plasmids, encodes an efflux mechanism that contributes to tetracycline resistance in HI [21].

Furthermore, resistance to levofloxacin has been reported in several countries and the main mechanism has been investigated [22, 23]. The main resistance occurs by the mechanism Amino acid alterations in the topoisomerase II and I genes quinolone resistance-determining region (QRDR) are the main cause of fluoroquinolone resistance in HI. Although it hasn't been documented in HI, mutations that cause permeability abnormalities and the overexpression of efflux pumps can likewise impair quinolone susceptibility [24].

Similarly, it has been reported that cases of antibiotic resistance against cephalosporins occur in a number of European countries. Resistance mechanisms have been identified and are related to the TEM-1 and ROB-1 varieties of plasmid-mediated β-lactamases [25, 26]. Penicillin-binding protein 3 (PBP3) alteration, which results in decreased affinity to penicillins and cephalosporins, is the most significant β-lactam resistance mechanism in b-lactamase-negative ampicillin-resistant isolates (BLNAR). This is because ftsI gene mutations cause amino acid substitutions in the transpeptidase domain of PBP3 [27, 28].

Cases of the high prevalence of bacteria resistant to some antibiotics, caused by overuse of antibiotics and the outcome of patient non-compliance with taking antibiotics, occurred in Taiwan [29-31]. Medical staff members, especially doctors and pharmacists, conduct extensive outreach initiatives to assist patients in taking their medicines as prescribed. Coordination between medical actors and health policymakers has implications for monitoring the use of antibiotics properly and encouraging public awareness of antibiotic adherence. The implementation of policies and monitoring of sustainable use of antibiotics in city health centers greatly supports the success of government programs in the regions. Although this study has a relatively large number of bacterial isolates, it has limitations from serotyping data for the isolates. Research in several health centers in cities in Indonesia is needed to determine the resistance profile of HI bacteria more broadly in Indonesia.

Some countries have reported that the vaccines used to treat HI type b (Hib) are ineffective. In comparison to pneumococci, the relative prevalence of non-typeable HI has increased as a result of the use of pediatric pneumococcal conjugated vaccinations [32, 33]. Testing for resistance is indeed required to determine how successful the use of antibiotics is efficient. Furthermore, effective infection management can stop the spread of resistance and minimize HI infection-related mortality [34].

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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