Comparative study of cefixime and tetracycline as an evaluation policy driven by the antibiotic resistance crisis in Indonesia

Danni Ramdhani1*, Sri Agung Fitri Kusuma2, Dede Sediana3, A. P. Hilarius Bima2 & Ika Khumairoh2

Antibiotic resistance is a serious threat that occurs globally in the health sector due to increased consumption of inappropriate antibiotics. Guidelines for prescribing antibiotics for ARTIs have been issued in general practice to promote rational antibiotic prescribing. This study was conducted to compare the effectiveness of cefixime and tetracycline as a solution to improve monitoring of appropriate antibiotic use in the treatment of ARTIs. All stock isolates were rejuvenated first, and cultured on standard media and Kirby–Bauer disc diffusion method was used for susceptibility testing in accordance with the Clinical and Laboratory Standard Institute's (CLSI) recommendations. Identification of bacteria from a single isolate was carried out to determine which bacteria were resistant to cefixime and tetracycline. A total of 466 single isolates of bacteria were analyzed, which showed a percentage of resistance to cefixime 38.0%, and tetracycline 92.86%. Bacterial isolates were resistant to cefixime and tetracycline was a genus of Haemophilus, Streptococcus, Corynebacterium, Staphylococcus, and bordetella. Cefixime compared to tetracycline was proven to be superior in terms of the effectiveness of ARIs treatment.

Acute respiratory tract infections (ARTIs), which involve the upper or lower respiratory tract are among the most common problems in general medical practice in developing countries, including Indonesia. The use of antibiotics has become a mainstay in its treatment, but it is now recognized that the benefits are often accompanied by disadvantages (adverse drug reactions, ineffective financial costs, and antibiotic resistance) 1. Antibiotic resistance can occur naturally, or clinically, which is the ability of bacteria to resist the effects of antibiotics 2. Overuse of antibiotics can contribute to an increase in cases of antibiotic resistance. Antibiotic prescribing guidelines for ARTIs in general practice have been established to ensure proper and effective treatment 3. ARTIs are the mainly reason for prescribing antibiotics in adults, and often they are not properly prescribed 4. The presence and spread of antibiotic resistance has become an increasingly serious public health problem 5.

These resistant bacteria will disrupt the success of the treatment process, and the increased cost of treatment per individual will also result in the epidemic spread of antibiotic-resistant infections 6. The implementation of policies to limit the spread of resistant bacteria from patient to patient includes improving hospital hygiene, using vaccines, monitoring rational use of antibiotics, and using antibiotic combinations 7.

Cefixime is a third generation cephalosporin antibiotic with activity as a bacteride which is able to damage the bacterial cell wall by the mechanism of action through inhibition of penicillin binding proteins, and damage the peptidoglycone synthesis pathway. Cefixime is widely used in many countries because it has broad spectrum activity against all Gram positive and negative pathogenic bacteria and atypical organisms, e.g. mycoplasma and chlamydia 8.

Tetracycline antibiotics are widely known to have a broad spectrum of activity, act on a variety of Gram-positive and negative bacteria, spirochetes, obligate intracellular bacteria, and are also effective against protozoan parasites 9. Tetracycline treatment for symptoms of respiratory tract infections is an antibiotic of choice, especially for infections caused by the potential pathogens of Haemophilus influenzae and Diplococcus pneumoniae. Medical
recommendations are also given to patients with chronic airway obstruction receiving antibiotics, and also if they show symptoms of acute infection 10.

The study reported by Ramdhani et al., regarding the management of ARTIs patients in a public health center of Tasikmalaya, Indonesia, described a fairly serious case of antibiotic resistance. The use of several antibiotics showed decreased effectiveness with a high resistance value, including amoxicillin (70.25%), levofloxacin (50.0%), ciprofloxacin (43.03%) 11–13.

This study was conducted to evaluate the comparative effectiveness of cefixime and tetracycline through antimicrobial susceptibility testing (AST) in the empirical treatment of ARTIs. In addition, this study was also to determine the genus of total clinical isolates of patients who had resistance to cefixime and tetracycline antibiotics. Tests were carried out using stock isolates from patients obtained from previous studies.

Materials and methods

The test sample was a combined stock isolate from previous ARTIs research totaling 466 single bacterial isolates. This sample has been purified from bacterial contaminants, and rejuvenated.

The bacterial growth medium used was Mueller Hinton Agar (MHA) (Oxoid) with a concentration of 38 g/L, according to the guidelines from the CLSI 14.

Biochemical test materials for bacterial identification include Lactose (Merck), Mannose (Merck), Maltose (Merck), peptone (Oxoid), phenol red (Taylor), Kovac’s reagent (Bio-Rad), TSIA , methyl red (HiMEdia), α-naphthosil (Merck).

Rejuvenation and purification of clinical isolates.  The technique of rejuvenating clinical isolates was carried out using the scratch plate method. Clinical isolates from previous studies were rejuvenated on new MHA growth media, and incubated at 37 °C for 18 h. Colony morphology observations were carried out including color, colony structure, and different haemolytic and morphological characteristics 15.

Preparation of test bacteria suspension. The test bacterial suspension was prepared by inoculating the bacterial colony into a sterile physiological NaCl solution. The turbidity of the bacterial suspension should be made equal to the standard turbidity of 0.5 Mc Farland solution 16.

Antimicrobial susceptibility testing (AST). The Kirby–Bauer disc diffusion test was used to determine the sensitivity of antibiotics to bacterial isolates 17. This test can evaluate the effectiveness of antibiotics that are already resistant or are still sensitive by measuring the diameter of the inhibition zone. This testing technique is based on the diffusion principle through antibiotic paper disks. This test was carried out with 3 repetitions. Determination of the resistance value of the tested bacteria to cefixime and tetracycline were carried out by comparing the diameter of the inhibition zone with the standard diameter of the resistance zone formed 14. The value of the resistance level category can be seen in Table 1.

Identification and morphologic and biochemical characterization. The gram stain technique, with microscopic visualization at ×100 magnification was used to ascertain cellular morphology, and bacterial classification. After identifying the phenotype and cell colonies, a conventional biochemical test was carried out to determine the classification of the isolated bacteria according to the existing biochemical testing protocol 18. The conventional biochemical tests are: GS = Gram Staining; MT = Motility Test; MR = Methyl Red; SC = Simone Citrate; TSIA = Triple Sugar Iron Agar; OX = Oxidase; UR = Urease Test, XY = Xylose; CT = Catalase Test; VP = Voges-Proskauer; carbohydrate fermentation test (LAC = Lactose, MAN = Manose; MAL = Maltose; SAC = Saccharose); IND = Indole Test 19.

Results and discussion

Biochemical conventional identification.  Bacterial isolates were obtained from patients who had been confirmed by a doctor indicating that they had suffered from respiratory tract infections. Statistically showed that the number of bacterial isolates consisted of the genus Staphylococcus (35%), Streptococcus (29%), Bordetella (15%), Haemophilus (12%), and Corynoboternium (9%).

Biochemical identification were carried out on 466 stocks of bacterial isolates, identified as Gram-negative, Gram-positive, bacilli (rod-shaped), cocoid (rod-shaped), cocobacilli. Results analyzed bacteria, the biochemical conventional tests revealed 5 genus of bacteria: Bordetella, Corynebacterium, Staphylococcus, Streptococcus, Haemophilus (Table 2, Fig. 1).

Several studies have shown similar results that of bacteria from the genus Bordetella, Haemophilus, Corynebacterium 20–23. Similar study conducted by Chopra et al. showed that Streptococcus haemoliticus,

| Categories | Cefixime (mm) | Tetracycline (mm) |
|------------|---------------|-------------------|
| Resistant  | ≤ 17          | ≤ 11              |
| Intermediates | 18–20       | 12–14              |
| Sensitives | ≥ 21          | ≥ 15              |

Table 1. Categories of cefixime and tetracycline inhibition zone diameter.
Staphylococcus, and Corynebacterium diphtheriae against tetracycline antibiotics 9. Cefexime is also reported to have started decreasing susceptibility patterns of these bacterial pathogens, Streptococcus pneumoniae, Hemophilus influenzae with low resistance levels. 24. Schico GC reported relevant information that cefexime showed decreased sensitivity to Staphylococcus, and streptococcus but was still very active than cefaclor and cefuroxime against Gram-negative respiratory pathogens 25.

However, some authors still highly recommend cefexime as a first-line antibiotic in overcoming cases of resistance to URTI and LRTI, especially against the pathogens Streptococcus pneumoniae, Streptococcus pyogenes, H. influenzae, Moraxella catarrhalis 26–28. Other studies have also demonstrated good clinical efficacy data of cefixime in URTI and acute otitis media (AOM) cases, where community-induced infections exhibit high rates of resistance to macrolides and are highly sensitive to cefixime 29,30.

Antibiotic susceptibility profile. The main focus of our study was to evaluate the current prevalence of bacteria responsible for ARTIs among the population in the urban area of Tasikmalaya, Indonesia. Our study investigated the correlation between pathogenic bacteria causing ARTIs and the antibiotic susceptibility profile commonly used by medical practitioners in public health centers. The test results obtained can be a comparative study regarding the effectiveness of cefixime and tetracycline as drugs of choice in the treatment of ARTIs. The disk antibiotic concentration used in accordance with CLSI guidelines are cefixime 5 µg, and tetracycline 30 µg14.

Antibiotic susceptibility test carried out on 466 bacterial isolate stocks showed that cefixime was superior to tetracyclines in the treatment of ARI. Cefixime showed a lower resistance level of 38.0% when compared to 92.8% tetracyclines. The level of tetracycline resistance was obtained from the number of bacteria genus Staphylococcus (158), Streptococcus (121), Bordetella (64), Hamophylus (52), and Corynobacterium (37). In addition, the value of the cefixime resistance level was derived from the number of each bacterial genus Staphylococcus (69), Streptococcus (45), Bordetella (27), Hamophylus (21), and Corynobacterium (15).

Additionally, the chi-square test was 308.0892, with the p-value was < 0.00001 and the result was significant at p < 0.05. These statistical data provide information for comparisons of resistant and non-resistant conditions from cefixime and tetracycline antibiotics described a significant difference in the level of resistance in the use of the two antibiotics for the treatment of ARTIs. These results are also consistent with other studies that cefexime is more effective in the treatment of respiratory infections compared to other drugs (eg ofloxacin, amoxicillin, and ciprofloxacin) 24,31. The susceptibility of categories of isolates of both antibiotics are shown in Fig. 2.

These results will provide important information to the Health Office of the City of Tasikmalaya to determine the antibiotic procurement policy for public health centers, and evaluate the pattern of prescribing antibiotics for ARTIs treatment by practicing doctors. Synergic cooperation and coordination between all health professions in the prevention of ARI is also very necessary.

Coordination with local policy holders of the Tasikmalaya City Health Office regarding the evaluation of the use of antibiotics in the treatment of ARTIs has been carried out. Preventive policies and education such as the intervention of antimicrobial stewardship programs (ASPs) at reducing unnecessary antibiotic prescribing, including training communication between health professionals, accountable justification of health programs, feedback by comparing socio-behavioral responses through questionnaires. Making handouts that are distributed

### Table 2. Results of biochemical test.

| Group of bacteria | Shape                | Gram  | Genus          | Biochemical test (positive) |
|-------------------|----------------------|-------|----------------|------------------------------|
| 1                 | Coccobacillus-capsulated | Negative | Bordetella | OX, CT, UR                   |
| 2                 | Bacilli-rod shaped    | Positive | Corynebacterium | MR, CT, TSIA, LAC, MAN, MAL   |
| 3                 | Round-shaped          | Positive | Staphylococcus | CT, MR, VP, UR, SC, LAC, MAN, MAL |
| 4                 | Cococcus-capsulated   | Positive | Streptococcus | LAC, MAL                     |
| 5                 | Cocccobacilli         | Negative | Haemophilus   | CT, OX, MAL                   |

Figure 1. Total percentage of cefexime and tetracycline resistance test against bacterial isolates.

Staphylococcus, and Corynebacterium diphtheriae against tetracycline antibiotics 9. Cefexime is also reported to have started decreasing susceptibility patterns of these bacterial pathogens, Streptococcus pneumoniae, Hemophilus influenzae with low resistance levels. 24. Schico GC reported relevant information that cefexime showed decreased sensitivity to Staphylococcus, and streptococcus but was still very active than cefaclor and cefuroxime against Gram-negative respiratory pathogens 25.

### Table 2. Results of biochemical test.

| Group of bacteria | Shape                | Gram  | Genus          | Biochemical test (positive) |
|-------------------|----------------------|-------|----------------|------------------------------|
| 1                 | Coccobacillus-capsulated | Negative | Bordetella | OX, CT, UR                   |
| 2                 | Bacilli-rod shaped    | Positive | Corynebacterium | MR, CT, TSIA, LAC, MAN, MAL   |
| 3                 | Round-shaped          | Positive | Staphylococcus | CT, MR, VP, UR, SC, LAC, MAN, MAL |
| 4                 | Cococcus-capsulated   | Positive | Streptococcus | LAC, MAL                     |
| 5                 | Cocccobacilli         | Negative | Haemophilus   | CT, OX, MAL                   |

Figure 1. Total percentage of cefexime and tetracycline resistance test against bacterial isolates.
to patients in community health centers as part of a program to educate the public about the use of appropriate antibiotics can support the success of ARTIs therapy. We plan to measure the impact of these interventions in the near future.

Conclusions

Cefexime treatment has shown superior effectiveness compared to tetracyclines in the case of ARTIs. Cefexime can still be used as a front line antibiotic option in the management of ARTIs.

The study also reported the identification of pathogenic bacterial organisms causing ARTIs and antibiotic resistance in accordance with research reports from another bacterial group *Bordetella*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Haemophilus*.

References

1. Smith, S., Fahey, T., Smucny, J. & Becker, L. Antibiotics for acute bronchitis. *Cochrane Database Syst. Rev.* 3, 1–19 (2014).
2. Nolte, O. Antimicrobial resistance in the 21st century: A multifaceted challenge. *Protein Pept. Lett.* 21, 330–335 (2014).
3. WHO. Antimicrobial Resistance Fact Sheet No. 194 (WHO, 2020).
4. Goossens, H. et al. Outpatient antibiotic use in Europe and association with resistance: Across-national database study. *Lancet* 365, 579–587 (2005).
5. Harris, A. M., Hicks, L. A. & Qaseem, A. Appropriate antibiotic use for acute respiratory tract infection in adults: Advice for high-value care from the American College of Physicians and the Centers for Disease Control and Prevention. *Ann. Intern. Med.* 164, 425–434 (2016).
6. Jacoby, G. A. Antimicrobial-resistant pathogens in the 1990s. *Ann. Rev. Med.* 47, 169–179 (1996).
7. Jernigan, D. B., Cetron, M. S. & Breiman, R. F. Minimizing the impact of drug-resistant *Streptococcus pneumoniae* (DRSP). *J. Am. Med. Assoc.* 275, 206–209 (1996).
8. Mandell, L. A. et al. Infectious diseases society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin. Infect. Dis.* 44, 27–72 (2007).
9. Chopra, I. & Roberts, M. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* 65, 232–260 (2001).
10. Woodhead, M. et al. Guidelines for the management of adult lower respiratory tract infections—Summary. *Clin. Microbiol. Infect.* 17, 1–24 (2011).
11. Ramdhani, D., Kusuma, S. A. F., Afifi, M. & Mustarichie, R. Amoxillin resistance in the area of Tasikmalaya, West Java. *J. Chem. Pharm. Res.* 8, 873–878 (2016).
12. Ramdhani, D. et al. Ciprofloxacin resistance among clinical isolates from acute respiratory infections (ARIs) patient at Community Health Centers in Tasikmalaya, Indonesia. *Asian J. Pharm. Clin. Res.* 10, 42–45 (2017).
13. Ramdhani, D., Kusuma, S. A. F., Azizah, S. N. & Sediana, D. Antibiotic resistance: Evaluation of levofloxacin treatment in acute respiratory tract infections cases at the Tasikmalaya City Health Center, Indonesia. *J. Adv. Pharm. Technol. Res.* 11, 113–116 (2020).
14. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. *CLSI Supplement M100* 27th edn, 1–15 (Clinical and Laboratory Standards Institute, 2017).
15. Kar, A. *Pharmaceutical Microbiology* 382 (New Age International Pvt Ltd, 2008).
16. The United State Pharmacopeial Convention. *The United States Pharmacopeia (USP)* 37th edn, 79–82 (The United State Pharmacopeial Convention, 2014).
17. Bauer, A. W., Kirby, W. N., Sheris, J. C. & Tuck, M. Antibiogram susceptibility testing by standardised single disc method. *Am. J. Clin. Pathol.* 36, 493–496 (1966).
18. Barrow, G. J. & Feltham, R. K. A. *Cowan and Steel’s Manual for the Identification of Medical Bacteria* 3rd edn. (Cambridge University Press, 1993).
19. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, I. T. & Williams, S. T. *Bergey’s Manual of Determinative Bacteriology* 9th edn, 457–678 (CABI, 1994).
20. Matteo, S. & Cherry, J. D. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. *Clin. Microbiol. Rev.* 18, 326–382 (2005).
21. Moxon, E. R. & Murphy, F. T. Haemophilus influenzae. In *Principles and Practice of Infectious Diseases* 6th edn (eds Mandell, G. L. et al.) 2369–2378 (Elsevier, 2005).
22. Van Roeden, S. E., Thijsen, S. F., Sankatsing, S. U. C. & Limonard, G. J. M. Clinical relevance of Corynebacterium pseudodiphtericum in lower respiratory tract specimens. *Infect. Dis.* 47, 862–868 (2015).
23. Varon, E. et al. Impact of antimicrobial therapy on nasopharyngeal carriage of *Streptococcus pneumoniae, Haemophilus influenzae, and Branhamella catarrhalis* in children with respiratory tract infections. *Clin. Infect. Dis.* 31, 477–481 (2000).
24. Ige, O. M. & Okesola, A. O. Comparative efficacy and safety of cefixime and ciprofloxacin in the management of adult with community-acquired pneumonia in Ibadan, Nigeria. *Ann. ib. Postgrad. Med.* 13, 72–78 (2015).
25. Schito, G. C., Georgopoulous, A. & Prieto, J. Antibacterial activity of oral antibiotics against community-acquired respiratory pathogens from three European countries. *J. Antimicrob. Chemother.* 50, 7–11 (2002).
26. Hedrick, J. A. Community-acquired upper respiratory tract infections and the role of third-generation oral cephalosporins. *Expert. Rev. Anti Infect. Ther.* 8, 15–21 (2010).
27. Hsueh, P. R. et al. Multicenter surveillance of antimicrobial resistance of *Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis* to 14 oral antibiotics. *J. Formos. Med. Assoc.* 103, 664–670 (2004).
28. Zafar, A. et al. Antibiotic susceptibility of pathogens isolated from patients with community-acquired respiratory tract infections in Pakistan—The active study. *J. Ayub. Med. Coll. Abbottabad.* 20, 7–9 (2008).
29. Negri, M. C., Morosini, M. I., Loza, E. & Baquero, F. Perspectives of oral cephalosporins in upper respiratory tract infections. *Clin. Microbiol. Infect.* 6, 56–58 (2000).
30. Principi, N. Oral cephalosporins in the treatment of acute otitis media. *Clin. Microbiol. Infect.* 6, 61–63 (2000).
31. Ullah, B., Ahmed, S., Shahariar, M. & Yesmine, S. Current trend of antibiotic resistance in lower respiratory tract infections (LRTIs): An experience in a teaching hospital in Bangladesh. *Bangladesh Pharm. J.* 19, 85–91 (2016).

**Acknowledgements**
The author thanks to Hilarius B.A.P and Ika Khumairoh for their contribution in this research.

**Author contributions**
D.R.: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper. S.A.F.K.: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data. I.K. and A.P.H.B.: worked the experiments. D.S.: Provided the medicine (antibiotics) that used at the health community centers.

**Competing interests**
The authors declare no competing interests.

**Additional information**

**Correspondence** and requests for materials should be addressed to D.R.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit [http://creativecommons.org/licenses/by/4.0/](http://creativecommons.org/licenses/by/4.0/).

© The Author(s) 2021