Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

Comparative Analysis of Azithromycin Resistance in Gram Positive Bacteria Pre- and Post- COVID-19 Pandemic

Hajir Qasim Razaq Alhusaynat ,Thi-Qar University / College Of Medicine

Abstract

Background: On the first day of the SARS-CoV2 pandemic, azithromycin (AZM) was one of the most commonly utilised drugs, even though there is no scientific evidence to support its usage in treating COVID-19. Because it encourages the establishment of drug-resistant bacterial strains, which makes it more tough to successfully treat a range of bacterial infections, its free egress has generated worries about public health. Perfect for learning how to combat COVID-19 infections using antibiotics that are effective against azole-group fever (AZM)-resistant bacteria.

Methods: In order to mimic cases of tonsillitis, pharynx infections, or respiratory tract infections, a control group consisting of 25 samples were used from healthy individuals as a control group and 37 bacterial isolates was maintained apart from cases that had previously contracted the corona virus. Ten scholarly articles were completely reviewed. All extra criteria were met by studies published between 2015 and 2023 that dealt with AZM resistance in bacteria during the treatment of bacteriosis or the fight against COVID-19.

Results: indicated that out of 25 control samples, 13 (or 52% of the total) responded positively to AZM whereas 12 (or 48% of the total) were resistant. In addition, 26 (70.27%) of the bacteria that were isolated were completely resistant to the antibiotic, whereas 11 (29.72%) of the isolates were found to be susceptible.

Conclusions: Implicit bacterial resistance to AZM is directly correlated with the careless use of this medication. Because AZM, an antibiotic drug, is widely used to treat a variety of bacterial diseases, the emergence of resistance bacteria poses a significant threat to public health.

Keywords: COVID-19; drug resistance; Azithromycin; Antibacterial.

Introduction : In December 2019, the first cases of the novel coronavirus, or nCoV, were discovered in China. The virus rapidly spread to neighboring nations (1). Consequently, On January 30, 2020, the World Health Organization (WHO) declared a Public Health Emergency of International Concern (PHEIC) in relation to the outbreak, which it later classified as a pandemic. More than 2 million persons in the European Region have died from complications since the COVID-19 epidemic was first detected (2). We have seen an increase in the introduction of tone-drug by individuals in this medium, which is hourly reported by the spread of bogus news, usually without any scientific foundation, on social and broadcast media. Azithromycin (AZM) was thereafter one of the most tone-specific medications employed in an alleged battle against COVID-19 (3). Bacterial resistance to this medication and its class increased as a result of the widespread usage of this medication without a scientific base that justifies choosing this medicine (4).

The azalide category of antibiotics includes the broad-spectrum macrolide AZM, which is derived from erythromycin. In terms of efficacy, it is highly active against gram-positive bacteria and quite weak against gram-negative ones (5). One form of lung infection that HIV-positive individuals frequently experience is disseminated Mycobacterium avium complex (MAC) infection, which can be treated or prevented with AZM. In addition to bacterial infections of the respiratory tract, skin, sinuses, ears, and reproductive organs, it is employed to treat bronchitis, pneumonia, and sexually transmitted diseases (6).

It works well against a variety of infections, including those caused by Moraxella catarrhalis, Hemophilus influenzae, Mycobacterium avium, Legionella pneumophila, Chlamydia trachomatis, and Streptococcus pneumoniae (7). AZM is a member of the class of medications known as macrolide antibiotics. It works by stopping the growth of germs. If you take antibiotics when they are not needed, you run the risk of developing antibiotic-resistant diseases later in life(8).

AZM's effect becomes a public health concern in this context because it encourages the establishment of drug-resistant bacterial strains, which reduces the efficacy of treating multiple bacterioses (7).

The ability to address common illnesses remains at risk due to the emergence and proliferation of drug-resistant bacteria that have evolved new mechanisms of resistance, known as antimicrobial resistance (9). The increasing number of patients experiencing treatment failures or succumbing to infections will escalate without effective prevention strategies and adequate treatment options for drug-resistant infections, alongside improved access to both new and established quality-assured antimicrobials (10).

Like other macrolide antibiotics, the primary goal of AZM is to specifically target the 50S component of the sensitive bacterial ribosome, thereby restricting bacterial protein synthesis; an increase in macrolide concentration is correlated with a decrease in protein synthesis (11).

Similar to other medications, it has been assumed that the primary factor contributing to the emergence of resistance bacteria in AZM is its poor use. Resistance organisms arise and proliferate when an incorrect dosage or length of treatment is administered (12). Gnococcal resistance against AZM has typically incorporated two strategies: Mutations in the mtrR coding area led to the overexpression of the MtrCDE efflux pump. Moreover, N. gonorrhoeae has a lower affinity for AZM due to changes in the genes generating the 23S rRNA component (13). Drug target alteration is associated with methylation of the 23S ribosomal subunit (associated with erm genes) or mutations in the rrl alleles of the 23S rRNA gene, which prevent macrolide binding to this subunit (12).

Aim of the Study: Given the over, the ideal of this study to understand the implications of bacterial resistance to azithromycin in the treatment of bacterioses, in the environment of the fight against COVID- 19.

Material and methods : This study included 62 participates who were classified into 37 that were diagnosed with tonsilitis, pharyngitis , respiratory tract infections and COVID-19. Patients , their ages ranged between 35 and 67 years. Whereas, 25 uninfected with COVID-19 subjects but had bacterial infections as controls, their ages ranged between 30 and 63 years.

All cases of the study were exposed to the same laboratory examinations to detect bacteria caused infections in tonsilitis, pharyngitis and respiratory tract and used the antibiotic AZM.

Culture media were prepared according to the manufacturer's instructions and sterilized using moist heat sterilization in an autoclave at 121°C and 15 pounds for 15 minutes. The sterilized media were then dispensed into sterile Petri dishes or test tubes, as required. To confirm sterility, the media were incubated at 37°C for 24 hours. The laboratory-prepared media, including Human Blood Agar, MacConkey Agar, and Mueller-Hinton Agar, were prepared following the protocol outlined by Magiorakos, et al (14).

The identification of bacterial isolates was performed based on their morphological characteristics on culture media, microscopic examination, and a series of biochemical tests.

Morphological Identification: Different media were used to detect and determine some specific characteristics of bacterial isolates. The size, shape, colour, and blood haemolysis pattern of the colonies cultivated on blood agar plates were examined. In order to evaluate each bacterial isolate's capacity to proliferate and ferment lactose sugar, the current study's isolates were all cultivated on MacConkey agar (15).

Microscopic Identification: After being transferred on a tiny slide, an isolated colony was fixed and stained with Gramme dye.

Bacterial Antibiotics Susceptibility Estimation : In vitro measurement of the ability of an antibiotic to inhibit bacterial growth is performed using the diffusion test which include that the paper discs that should be impregnated with antibiotic are placed on agar medium uniformly seeded with the tested bacteria. According to the sensitive or resistance of the tested bacteria, the area around each antibiotic disc suggested to be clear as a sign to bacterial inhibiting via the antibiotic , commonly termed as (IZ) which their diameters are differ according to the bacterial susceptibility for the certain antibiotic (16).

At first five ml of normal saline was inoculated with the tested bacterial isolate to form diluted suspension for each tested isolate. Then by using Sterilized swabs, bacterial suspensions were cultivated on Mueller Hinton agar plates. Antibiotic discs of azithromycin were placed onto the cultivated plates using a pair of tweezers. The plates were incubated at 37 Co under aerobic condition for 24-48 hours. Following incubation, the results were compared to the universal antibiotics manual to determine if they were Susceptible (S), Intermediate (I), or Resistant (R).

Results: The present study showed that bacteria resistance was more evident in covid-19 patients 70.27%, while in controlled patients' bacteria resistance approximately 48%|. But all samples, whether they were infected with Covid-19 or healthy people, had bacterial growth.as shown in Table 1-1.

Table (1-1) Subjects of the present study

Total Samples	No. Of Samples	No Bacterial Growth	Bacterial Growth
Patients	**37**	**0**	**37**
Control	**25**	**0**	**25**

Antimicrobial resistance was demonstrated in the present study by using disk diffusion method and one type of antibiotics were used. The table below (Table 1-2) showed the resistance of bacteria isolated from patients previously infected with the Corona virus and who used within the protocol the antibiotic AZM treatment, as well as bacteria isolated from patients with respiratory diseases but not infected with the Corona virus.

Table (1-2): Bacterial isolates of the study and their antibiotic resistance classification

Type Of Sample	No. Of Isolation	Name Of Antibiotic & Dose	Sensitive	Resistant
		AZM		
No. Isolates Bacteria from Covid-19 Patients	37	15 µg	11 (29.72%)	26 (70.27%)
No. Isolates Bacteria from Uninfected with Covid-19	25	15 µg	13(52%)	12(48%)

Discussion: Since antibiotics select the most resistant germs through a variety of mechanisms of action, it is normal for some bacteria to develop resistance to their effects over time (17). This is a natural biological process that has occurred over billions of years without human intervention as a result of microorganisms being exposed to antimicrobial compounds produced by other microbes. Resistance genes are selected for in this way, and these genes can then be passed on to human pathogens (18). Consequently, the bacterium develops resistance to antimicrobial agents through physiological processes. One of these needs to be considered: plasmids, which are little pieces of DNA that can replicate genetically and transfer resistance from one bacterium to another by conjugation, transformation, transduction, or transposition, expose bacteria to drugs through genetic mutation. Additionally, efflux pumps and porins are defensive mechanisms present in bacterial cells (19).

The main way that bacteria develop resistance to AZM is by shifting the position of the antibiotic's active component on the ribosome. This is caused by the bacterial erm gene demethylating the 23S subunit's adenine nucleotide and changing its conformation 12, which stops the macrolide from attaching to the ribosome and the peptide chain from translocating (20).

Table 1-2 discloses bacterial resistance to AZM treatment before and after COVID-19 infection. We noticed that 26 out of 37 patients with Covid-19 had bacterial resistance to AZM drug of approximately (72%). While the number of people who were not infected with Covid-19 was resistant to treatment by bacteria, it was approximately (48%).

There are specific streptococci and staphylococci that produce proteins that enable the release of macrolides from the cytoplasm. This results in a decrease in the effective concentration of antibiotics within the cell, which in turn hinders the capacity of antibiotics to operate (21). In the cell membranes of Gram-negative bacteria, the presence of specific pore proteins that inhibit AZM from accessing the cytoplasmic space is less likely to be present by these bacteria (22).

The selection of bacterial strains with long-standing genetic traits that did not multiply in subsequent strains due to lack of adaptive benefits can be attributed to the widespread use of antibiotics (23). Since the medication primarily affects sensitive lines, the resistance trait's genetic transmission will be more noticeable in subsequent generations, creating a population that is resistant to the medication (24).

A stronger correlation has been shown between widespread usage of AZM and bacterial resistance to the antibiotic during COVID-19, when certain individuals in the medical community and general public utilize the drug for self-medication without a legitimate scientific justification (25).

The majority of the time, AZM was found to be unnecessary, demonstrating the medication's indiscriminate prescription, which exacerbates bacterial resistance to the antibiotic in question. AZM was given in 59% of COVID-19 hospitalizations, even in cases where there was no bacterial co-infection, whereas only 15% of SARS-CoV2 infections result in a bacterial co-infection that warrants the administration of antibiotic therapy (26). The Antimicrobial Stewardship Program (ASP) and directives from the Ministry of Health (MOH) or World Health Organization (WHO) should be closely followed while prescribing antibiotics (27).

Conclusion: medicine-resistant bacterial strains were more likely to emerge since many of the individuals who were approved to use AZM to treat COVID-19 did not have a concurrent bacterial infection that would have justified its use. As a result, the medicine was given to them without the required medical rationale. In this instance, a clear connection between the outcomes of careless AZM use may be demonstrated. Further laboratory research is required to evaluate the level of resistance that various bacterial strains have acquired against the antibiotic action of AZM. Antibiotic stewardship initiatives must therefore be put in place in order to reduce the possibility of antibiotic resistance developing. Such programs should be predicated on the following principles: To minimize needless antibiotic exposure, (1) covid-19 should not be treated (with AZM); (2) antibiotics should be used at the right doses, not at the wrong dosages, to potentially prevent the creation of mutants; and (3) antibiotics should be used for the right length of time to reduce recurrence. Furthermore, regulations or self-medication should restrict the availability of over-the-counter (OTC) medications in order to minimize antibiotic overuse. Lastly, nonantimicrobial prophylaxis could be used to successfully lower the overall quantity of antibiotic usage.

I would like to extend my thanks and gratitude to Bint Al-Huda Hospital and the Al-Turki Hospital and their staff for their cooperation and approval to conduct the research in one of the hospital’s laboratories.

I would like to express my gratitude towards my friends in developing the research and people who have willingly helped me out with their abilities

References

1. Park, S. E. (2020). Epidemiology, virology, and clinical features of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2; Coronavirus Disease-19). Clinical and experimental pediatrics, 63(4), 119. doi: 10.3345/cep.2020.00493
2. Yüce, M., Filiztekin, E., & Özkaya, K. G. (2021). COVID-19 diagnosis—A review of current methods. Biosensors and Bioelectronics, 172, 112752. doi.org/10.1016/j.bios.2020.112752
3. Sultana, J., Cutroneo, P. M., Crisafulli, S., Puglisi, G., Caramori, G., & Trifirò, G. (2020). Azithromycin in COVID-19 patients: pharmacological mechanism, clinical evidence and prescribing guidelines. Drug safety, 43, 691-698. doi.org/10.1007/s40264-020-00976-7
4. Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. Nature reviews microbiology, 13(1), 42-51. doi.org/10.1038/nrmicro3380
5. Ranjbar, R., & Alam, M. (2024). Antimicrobial Resistance Collaborators (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Evidence-Based Nursing, 27(1), 16-16. doi.org/10.1136/ebnurs-2022-103540
6. Wahab, S., Ahmad, M. F., Hussain, A., Usmani, S., Shoaib, A., & Ahmad, W. (2021). Effectiveness of Azithromycin as add-on Therapy in COVID-19 Management. Mini Reviews in Medicinal Chemistry, 21(19), 2860-2873. doi.org/10.2174/1389557521666210401093948.
7. Bakheit, A. H., Al-Hadiya, B. M., & Abd-Elgalil, A. A. (2014). Azithromycin. Profiles of drug substances, excipients and related methodology, 39, 1-40. doi.org/10.1016/B978-0-12-800173-8.00001-5
8. Heidary, M., Ebrahimi Samangani, A., Kargari, A., Kiani Nejad, A., Yashmi, I., Motahar, M., ... & Khoshnood, S. (2022). Mechanism of action, resistance, synergism, and clinical implications of azithromycin. Journal of clinical laboratory analysis, 36(6), e24427. doi.org/10.1002/jcla.24427
9. Chiou, C. S., Hong, Y. P., Wang, Y. W., Chen, B. H., Teng, R. H., Song, H. Y., & Liao, Y. S. (2023). Antimicrobial resistance and mechanisms of azithromycin resistance in nontyphoidal Salmonella isolates in Taiwan, 2017 to 2018. Microbiology spectrum, 11(1), e03364-22. doi: 10.1128/spectrum.03364-22. Epub 2023 Jan 23.
10. Majumder, M. A. A., Rahman, S., Cohall, D., Bharatha, A., Singh, K., Haque, M., & Gittens-St Hilaire, M. (2020). Antimicrobial stewardship: fighting antimicrobial resistance and protecting global public health. Infection and drug resistance, 4713-4738. doi: 10.2147/IDR.S290835
11. Parnham, M. J., Haber, V. E., Giamarellos-Bourboulis, E. J., Perletti, G., Verleden, G. M., & Vos, R. (2014). Azithromycin: mechanisms of action and their relevance for clinical

applications. Pharmacology & therapeutics, 143(2), 225-245. doi: 10.1016/j.pharmthera.2014.03.003.

12. Unemo, M., Golparian, D., & Eyre, D. W. (2019). Antimicrobial resistance in Neisseria gonorrhoeae and treatment of gonorrhea. Neisseria gonorrhoeae: methods and protocols, 37-58. doi: 10.1007/978-1-4939-9496-0_3.

13. Doan, T., Worden, L., Hinterwirth, A., Arzika, A. M., Maliki, R., Abdou, A., ... & Lietman, T. M. (2020). Macrolide and nonmacrolide resistance with mass azithromycin distribution. New England Journal of Medicine, 383(20), 1941-1950. doi: 10.1056/NEJMoa2002606.

14. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection, 18(3), 268-281. doi: 10.1111/j.1469-0691.2011.03570.x.

15. Kuleshova, S. I., Protsak, S. A., Lisunova, S. A., & Romanyuk, G. Y. (2021). Stability of ready-to-use and laboratory-prepared culture media. Regulatory Research and Medicine Evaluation, 11(2), 130-134. doi.org/10.30895/1991-2919-2021-11-2-130-134

16. Tibbits, G., Mohamed, A., Call, D. R., & Beyenal, H. (2022). Rapid differentiation of antibiotic-susceptible and-resistant bacteria through mediated extracellular electron transfer. Biosensors and Bioelectronics, 197, 113754. doi: 10.1016/j.bios.2021.113754.

17. Huemer, M., Mairpady Shambat, S., Brugger, S. D., & Zinkernagel, A. S. (2020). Antibiotic resistance and persistence—Implications for human health and treatment perspectives. EMBO reports, 21(12), e51034. doi: 10.15252/embr.202051034.

18. Larsson, D. G., & Flach, C. F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology, 20(5), 257-269. doi: 10.1038/s41579-021-00649-x.

19. Miranda, C., Silva, V., Capita, R., Alonso-Calleja, C., Igrejas, G., & Poeta, P. (2020). Implications of antibiotics use during the COVID-19 pandemic: present and future. Journal of Antimicrobial Chemotherapy, 75(12), 3413-3416. doi: 10.1093/jac/dkaa350.

20. Rufino, J. P., Sousa, L. G. V., Rodrigues, A. E. L., Falcão, A. L. S., de Melo Lima, I. C., de Miranda Cavalcanti, L. A., ... & de Oliveira Filho, J. W. G. (2022). Bacterial resistance to azithromycin: causes, effects, and the fight against COVID-19. Research, Society and Development, 11(6), e27711629198-e27711629198. doi.org/10.33448/rsd-v11i6.29198

21. Crespi, E., Pereyra, A. M., Puigdevall, T., Rumi, M. V., Testorelli, M. F., Caggiano, N., ... & Srednik, M. E. (2022). Antimicrobial resistance studies in staphylococci and streptococci isolated from cows with mastitis in Argentina. Journal of Veterinary Science, 23(6). doi: 10.4142/jvs.21062.

22. Mupfunya, C. R., Qekwana, D. N., & Naidoo, V. (2021). Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga, South Africa. Veterinary Medicine and Science, 7(1), 112-121. doi: 10.1002/vms3.334.

23. Septimus, E. J. (2018). Antimicrobial resistance: an antimicrobial/diagnostic stewardship and infection prevention approach. Medical Clinics, 102(5), 819-829. doi: 10.1016/j.mcna.2018.04.005.
24. Christaki, E., Marcou, M., & Tofarides, A. (2020). Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. Journal of molecular evolution, 88(1), 26-40. doi: 10.1007/s00239-019-09914-3.
25. Retallack, H., Di Lullo, E., Arias, C., Knopp, K. A., Laurie, M. T., Sandoval-Espinosa, C., ... & DeRisi, J. L. (2016). Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proceedings of the National Academy of Sciences, 113(50), 14408-14413. doi: 10.1073/pnas.1618029113.
26. Singh, N., Tang, Y., Zhang, Z., & Zheng, C. (2020). COVID-19 waste management: Effective and successful measures in Wuhan, China. Resources, conservation, and recycling, 163, 105071. doi: 10.1016/j.resconrec.2020.105071.
27. Sulayyim, H. J. A., Ismail, R., Hamid, A. A., & Ghafar, N. A. (2022). Antibiotic resistance during COVID-19: a systematic review. International journal of environmental research and public health, 19(19), 11931. doi: 10.3390/ijerph191911931.