JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EMERGING AZITHROMYCIN AND CEFTRIAXONE RESISTANCE AMONG BACTERIAL ISOLATES FROM GROUNDWATER

Pratima Wagh[1], Amruta Jadhav [2]
Department of Microbiology, M.V.P. Semaj's, K.T.H.M College, Shivajinagar, Gangapur road, Nashik- 422002, Maharashtra, India

Abstract: During COVID 19 pandemic, the patients having milder forms of respiratory tract infections received azithromycin and ceftriaxone. These antibiotics are ineffective in treatment of viral infections such as common cold and flu but active against many bacterial infections of various systems in the body. Misuse of such broad-spectrum antibiotics can lead to its decreased effectiveness. There is evidence of traces of antibiotics in groundwater leading to development of increased resistance among the microorganisms. Present study was on Azithromycin and ceftriaxone resistant isolates obtained from water samples from Nasik city of Maharashtra. From June 20 there was a sharp rise in the number of COVID cases, reaching more than 90,000 in the month of September in Nashik region. There is a confirmatory report of contamination of groundwater by the leachate from waste dumps in Nasik in previous studies. Considering the scenario, from groundwater samples, azithromycin and ceftriaxone resistant organisms were isolated and identified. These antibiotic resistant organisms were isolated from open wells and from river water samples, while isolates from other sources are found sensitive to both of these drugs.

Index Terms - Azithromycin, Ceftriaxone, Bacterial resistance, Groundwater, COVID19

I. Introduction

Covid 19, a pandemic, expresses a varied range of illness, which progresses from asymptomatic to milder and then fulminant infection with multiorgan system failure. Strongly effective antivirals against corona viruses are not identified yet and therefore the majority of patients having milder forms of respiratory tract infections receive antibiotics like Azithromycin, Doxycycline and Ceftriaxone. All of these antibiotics are ineffective in treatment of common cold and flu causing viruses but active against many bacterial infections of various systems in the body. Azithromycin belongs to a class of antibiotics known as macrolides. It can be used to treat bacterial sinusitis, tonsillitis, ear infections, pneumonia and many others. WHO also reports that Azithromycin is being widely used with hydroxychloroquine although it is not yet recommended outside of COVID-19 clinical trials[1] Azithromycin and other macrolides have been largely used to treat Gram-positive infections and also possess good activity against different Gram-negative microorganisms, such as Bartonella spp., Campylobacter spp., Hemophilus influenzae and Neisseria gonorrhoeae [2]There are potential threats that could affect antimicrobial stewardship activities and drive antimicrobial resistance[1]Since antibiotic resistance is a severe health problem worldwide which can lead to inefficiency of antimicrobial agents and therapeutic failure[3] surveillance of the development of antimicrobial resistance should be performed, establishing molecular mechanisms of resistance to thereby design alternative treatments. Ceftriaxone belongs to a class of drugs known as cephalosporin antibiotics. It is used to treat infections such as urinary tract infections, skin infections, meningitis, lower respiratory tract infections caused by bacteria. It is bacteriostatic, and during the current pandemic, used for treatment of patients having cold like symptoms. Misuse of such broad-spectrum antibiotics can lead to its decreased effectiveness.

Present study was on gram positive and gram-negative isolates showing resistance to Azithromycin and ceftriaxone and were isolated from ground water samples from the city of Nasik, state of Maharashtra. From June 20 there was a sharp rise in the number of COVID cases, reaching more than 90,000 in the month of September in Nashik region. (Fig 1) There was a confirmatory report of contamination of groundwater by the leachate from waste dumps in Nasik in previous studies [4]

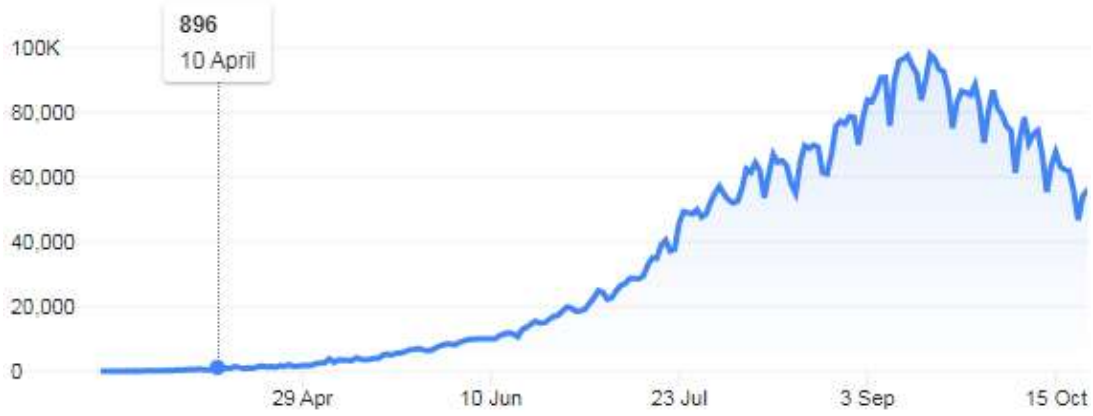


Fig:1: Cases of covid 19 in Nashik.

(Wikipedia, government health ministries, The New York Times, and other authoritative sources, as attributed)

Consequently, twenty water samples were collected from various sources of ground water such as from open wells, boar wells, water from hand pumps and from rivers. Five samples were collected from treated water in the distribution system as owing to misuse of antibiotics there is evidence of traces of antibiotics in groundwater leading to development of increased resistance among the microorganisms[5,6] As the main cause for development of antibiotic resistance is anthropogenic activities, current work may add valuable actualities by raising alertness amongst the society concerning ill use of antibiotics. Antimicrobial resistance is a neglected global crisis that requires urgent attention and action.

II Materials and Methods:

2.1: Study Design: Experimental Study

2.2: Study Period: June 2020 to December 2020

2.3: Study Centre: Department of Microbiology, MVP Semaj's K.T.H.M. College, Nashik, Maharashtra, India.

2.4: Sample size: Total 25 samples were collected from open wells, boar wells, water from hand pumps, river water and samples of treated water from distribution systems.

2.5: Sample collection: Using sterile screw capped bottle, and preserved under refrigeration.

2.6: Inclusion criteria: After disc diffusion testing for Azithromycin and Ceftriaxone resistant gram positive as well as gram negative isolates were identified.

2.7: Exclusion criteria: Organisms sensitive to Azithromycin and ceftriaxone.

2.8: Bacteriological testing of samples-

2.8.1: Inoculation of samples on nutrient agar plates using streak plate method. Incubation of plates at 37^0 C for 18- 24 hours.

2.8.2: After observing colony characteristics, selection and subculturing of the isolates on sterile nutrient agar slants. (Fig-2)

2.8.3: Antibiotic susceptibility testing was performed by using Kirby Bauer disc diffusion method[7] as per standard guidelines described by Clinical Laboratory Standard Institute. Fresh subcultures were inoculated on the Mueller-Hinton agar plate. Rings of filter paper discs impregnated with standardized concentration of antibiotics (by Dynamicro lab) were placed on the surface of agar plates and incubated overnight at 37^0C. Zone of inhibition was measured around the discs after incubation.

2.8.4: Observations of test results were recorded as per the guidelines by Dynamicro lab. The zone of inhibition was measured and recorded as resistant (R), sensitive(S), Intermediate(I) As a quality control stock culture of E. coli (ATCC 25922) and Staph aureus (ATCC 25923) were used.

Table:1 Standard chart of inhibition zones of bacteria by disc diffusion method as per Dynamicro laboratory ltd.

Gram Positive - Inhibition Zone [mm]				
Antibiotic	Concentration	Resistant	Intermediate	Sensitive
Azithromycin AZ	15 mcg	≤13	14 – 17	≥18
Gram Negative - Inhibition Zone [mm]				
Ceftriaxone CTX	30 mcg	≤13	14-20	≥21

mcg: microgram
mm: millimeter

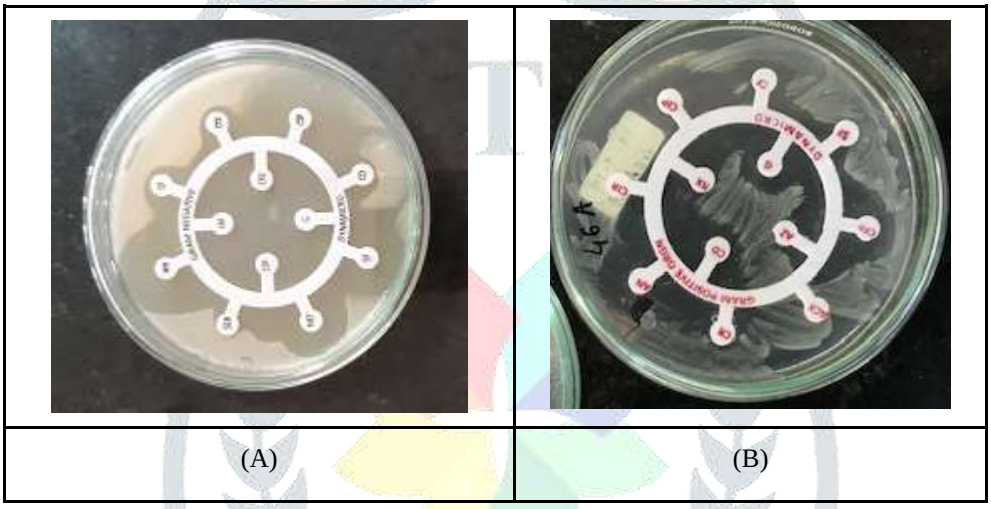
2.8.5: The Multi Discs from Dynamicro labs contain 15 mcg azithromycin to which resistance was observed, therefore testing is carried out for higher doses, i.e., 20, 30, 40, 50 mcg respectively, by using disc diffusion test. Similarly, Ceftriaxone content in Dynamicro test discs was 30 mcg, to which resistance was observed, therefore by using 40 mcg, 50 mcg and 60 mcg impregnated paper discs having ceftriaxone tests were carried out. Resistant strains for azithromycin and ceftriaxone were identified by cultural, biochemical characteristics as per Bergey's manual of determinative bacteriology.

III Results

3.1: Isolation of organisms: 46 isolates showing different colony characteristics were isolated.

Fig 2: Pure culture isolates from water samples.

3.2: Disc diffusion test using Multi Discs: Gram negative organisms isolated from open well (2C), from waldevi river water 45(A) 46(B) and 4(C)water from handpump showed resistance against ceftriaxone, similarly Gram-positive isolates no.10(B), 31(A), 46(A), 53(A) and 53(B) showed resistance against azithromycin.



(A) (B)

Fig 3: Resistance against Ceftriaxone (A) and Azithromycin (B)

Table:2 Zone Diameter for Ceftriaxone by Gram negative isolates.

Sr. No	Isolate Number	Zone Diameter for disc containing 30 mcg Ceftriaxone (CTX)
1	2C	6mm
2	4C	8mm
3	45A	R
4	46B	R

mcg: microgram
mm: millimeter
Table:3

3.4: Identification of organisms

3.4.1: Following tests for identification of gram-positive cocci were carried out: Catalase Test, Carbohydrate fermentation test, Test for yellow pigment production, 7.5% NaCl tolerance test, Starch hydrolysis test, Gelatin Test, Oxidase Test, Citrate utilization test, Tests for hemolysis using blood agar plates as per Bergey's manual of Systematic bacteriology.

Fig 4: Blood agar plates showing hemolysis/no hemolysis.

Table: 4 Identified Resistant gram-positive cocci shaped organisms

Sr. No	Isolate number	Identified organism
1	46(A),53(B)	*Staph aureus*
2	10(B)	*Micrococcus*
3	31(A)	*Streptococcus*

3.4.2: Tests for gram negative bacteria: Motility, Capsule staining, Lactose fermentation, Glucose utilization (A/G), IMViC Tests, Urease test, KCN. H2S production.

Table: 5 Identified Resistant gram-negative cocci shaped organisms

Sr. No	Isolate number	Identified organism
1	(45A)	*E. coli*
2	(2C)	*Salmonella*
3	46(B)	*Klebsiella*
4	4(C)	Proteus

3.5: Disc diffusion test using higher concentration of antibiotics.

3.5.1: Staphylococcus showed resistance to Azithromycin when tested for 20 mcg, 30mcg, 40 mcg having discs.

3.5.2: E. coli isolated from river water samples showed resistance for 40 mcg Ceftriaxone having paper disc.

IV. Conclusion and Discussion

The present study reveals resistance to Azithromycin and Ceftriaxone in microorganisms isolated from ground water samples. These drugs are widely used to treat patients having cold or flu like symptoms and for patients suffering from covid 19. By using disc diffusion method resistance of isolates was tested. After isolation of bacteria from the 25 samples, identification of resistant microorganisms was carried out using Bergey's manual of determinative bacteriology. Five gram positive and four-gram negative organisms were identified as resistant strains to azithromycin and ceftriaxone respectively. Zone of inhibition equal to or less than 13 mm for 30 mcg of ceftriaxone in standard conditions is considered resistant while a similar zone for 15 mcg of azithromycin is considered as resistant as per standard protocol provided by Dynamicro lab, the paper disc manufacturer laboratory in India. These nine isolates showed complete resistance (no zone of inhibition) and were isolated from open wells and from river water samples, while isolates from other sources are found sensitive to both of these drugs suggesting that development of resistance among the organisms in the untreated water. There may be horizontal gene transfer of antibiotic resistance gene pool. Among Gram negative isolates lactose fermenters such as *E. coli* found nearly in all samples except two treated water samples. While *Salmonella* spp, *Proteus* spp. and *Klebsiella* spp were identified mainly in open well and river water samples. Subsequently in the Gram-positive category majority of isolates are identified as the Staphylococcus species followed by Streptococcus and *Micrococcus* species.

Disk diffusion technique used in the study categorizes organisms in Resistant, Sensitive and Intermediate through quantitative results obtained, but at the same time indicate resistant mutants.

References

[1] Clinical management of COVID-19, Interim Guidance - May 2020. Geneva: World Health Organization; 2020. Available from: https://www.who.int/ publications-detail/clinical-management-of-covid-19

[2] Gomes C, Ruiz-Roldan L Mateu J, Ochoa TJ, Joaquim Ruiz, 2017. Macrolide resistance mechanisms in Enterobacteriaceae: Focus on azithromycin. *Crit Rev Microbiol*, 43 (1). Doi: 10.3109/1040841X.2015.1136261.

[3] Farrar WE, 1985. Antibiotic resistance in developing countries. *J Infect Dis*, 152 :1103 Doi: 10.1093/infdis/152.6.1103.

[4] Wagh Pratima Pandit, More S., 2018. Groundwater pollution by leachate using a case study of waste treatment plant at Nashik. *International Journal of Creative Research Thoughts*, 6: 2320.

[5] Z. Wang, Y. Du, C. Yang, X. Liu, J. Zhang, E. Li, X. Wang, 2017. Occurrence and ecological hazard assessment of selected antibiotics in the surface waters in and around Lake Honghu, China. *Sci Total Environ*, 609: 1423.

[6] A.R. Mahmood, H.H. Al-Haideri, F.M. Hassan, 2019. Detection of antibiotics in drinking water treatment plants in Baghdad city, *Iraq. Adv Public Health*, 10

[7] Bauer AW, Kirby WM, Sherris JC, Jurck M., 1996. Antibiotic susceptibility testing by a standardized single disc diffusion method. *Am J Clin Pathol*, 45: 493.
[8] Wagh Pratima, Patil G. M., Pawar I. S., Wendait M. R., Waghchaure R. B. 2020. Antibiotic resistance among gram positive isolates from an aquatic environment: a case study. *International Journal of Advanced Science and Technology*, *29*(7): 4620-4628. Retrieved from http://sersc.org/journals/index.php/IJAST/article/view/23326
[9] P.P. Wagh, V. S. Shinde, G.R. Chaudhari, I. L. Sayyed, T. U. Khapre, 2021. Antibiotic resistance patterns of gram-negative isolates from water: a case study of Nasik**.** International Journal of Multidisciplinary and Current Educational Research (IJMCER), 3 (2): 41-48.