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Original Article

Determination of Minimum Inhibitory Concentration of Different Antibiotics against Pseudomonas aeruginosa by E- test at a Tertiary Care Hospital

Mst. Romena Khatun,[1] Kh. Md. Faisal Alam,[2] Sabera Gul Nahar,[3] Nurjahan Begum,[4] Md. Khaled Shahrear,[5] Quazi Tamanna Hauqe [6]

Abstract

Background: Antimicrobial drug resistance is a matter of worldwide concern. Different microorganisms, including bacteria, are becoming more resistant day by day. So it is of utmost importance to know the antimicrobial susceptibility pattern not only for initiation of treatment but also for prevention of drug resistance. By determining minimum inhibitory concentration (MIC) by Epsilometer-test (E-test), we can determine the exact dose of a particular drug.

Aims: The study is aimed to determine the MIC of different antibiotics against intermediate isolates of *P. aeruginosa* isolated from clinically suspected patients of chronic suppurative otitis media (CSOM).

Materials and Methods: A cross-sectional type of descriptive study was conducted in the Department of Microbiology, Rajshahi Medical College, Rajshahi, during the period of January 2019 to December 2019 to identify the causative bacteria of CSOM with their antibiotic susceptibility pattern. A total of 96 aural swabs were collected from clinically suspected cases of CSOM, irrespective of age and sex, in the ENT department of Rajshahi Medical College Hospital, Rajshahi. Isolation and identification of bacteria were made as per standard procedure, and then antimicrobial susceptibility testing was performed by the disc diffusion method. At last, E-test was performed on different antibiotics against intermediate isolates of *P. aeruginosa.*

Results: Among 73 isolates, 37 showed intermediate susceptibility towards selected antibiotics such as beta-lactams (Ceftazidime, Ceftriaxone), aminoglycosides (Gentamicin), and quinolones (Ciprofloxacin) by disc diffusion method. Among 37 intermediate isolates, *P. aeruginosa* was 14 in number. Regarding MIC breakpoints in terms of susceptibility, out of 14 intermediate isolates of *P. aeruginosa,* 09(64.28%) isolates were susceptible, 03(21.43%) intermediate, and 2(14.28%) were resistant to different antibiotics by E-test.

Conclusion: In this study, higher susceptibility of different antibiotics against intermediate isolates of *P. aeruginosa* was observed by E-test than the disc diffusion method.

Key words: *Pseudomonas aeruginosa*, MIC, E-test.

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Introduction

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism following overnight incubation.[1] The lower the MIC, the better the bactericidal activity of a drug.[2]

According to the Clinical and Laboratory Standards Institute (CLSI, 2017), the results of

[1] Assistant Professor (C.C), Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh.
[2] Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh.
[3] Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh.
[4] Associate Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh.
[5] Assistant Professor (C.C), Department of ENT, Rajshahi Medical College, Rajshahi, Bangladesh.
[6] Lecturer, Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh.

AST are categorized as susceptible (S), intermediate (I), and resistant (R) on the basis of MIC breakpoints.[3] But according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019, the intermediate (I) category has been termed recently as 'I' - susceptible, increased exposure and is defined as a microorganism is categorized as susceptible, increased exposure when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of an infection.[4] Usually, the intermediate (I) category drug is considered resistant (R) by many clinicians during treatment. But by determining the MIC of the intermediate isolates, we can determine the exact dose of that particular drug. Thereby many common and cost-effective drugs can be used effectively.[5] In Bangladesh, the commonly used drugs against both Gram-positive and Gram-negative bacteria are usually beta-lactams, aminoglycosides, quinolones, and macrolides. Therefore, the determination of the MIC of these drugs against the intermediate isolates of CSOM will be helpful to evaluate the dose of a particular drug at which the organism will be susceptible or whether they are becoming resistant to that drug.

MIC can be determined by several methods such as broth dilution, agar dilution, Epsilometer-test (E-test), and Autobac method. Epsilometer-test (E-test) has been developed to provide a direct quantification of the antimicrobial susceptibility of microorganisms. MIC determining methods, like E-test, provide quantitative measurement of antimicrobial susceptibility.[5] Because of their cost and limited availability in developing countries, their application is not as frequently used as the disc diffusion method.[6,7] But, it has an extensive range of over 100 antimicrobial references that can be classified into four categories: antibiotics, antifungal, anti-mycobacterial, and resistance phenotype testing. E-test is better than other methods because it is easy to perform and requires minimal training for test performance, contamination can be easily recognized, can be easily set up for a small number of clinical isolates, and adequate method to detect potentially resistant strains of different organisms. It can also provide an exact MIC value, which allows fine-tuning of the antibiotic treatment regimens of patients.[8]

Materials and Methods

A cross-sectional type of descriptive study was conducted in the Department of Microbiology, Rajshahi Medical College, Rajshahi, during the period of January 2019 to December 2019. All patients, irrespective of age and sex, were the population with clinically suspected CSOM in the ENT department of Rajshahi Medical College Hospital, Rajshahi. The sample size was 96. First, aural swabs were collected as per the standard procedure. Then all the collected specimens were inoculated in nutrient agar, blood agar, and MacConkey's agar media and were incubated aerobically at 37^0C overnight (18-20 hours). Bacterial isolates were identified by microscopy, culture morphology, and relevant biochemical tests. Then antimicrobial susceptibility pattern was made by the disc diffusion method. MIC of selected antibiotics of intermediate isolates of *P. aeruginosa* was determined by E-test, and the results were noted.

Inclusion criteria:

1. Persistent or intermittent ear discharge over 12 weeks through a perforation of the tympanic membrane.
2. Patients of all age groups of both sexes.
3. Patients who were not on antibiotic therapy for the last three days prior to sample collection.

Exclusion criteria:

1. Acute suppurative otitis media.
2. Chronic otitis media with effusion.
3. Patients of CSOM with otitis externa.
4. Patients are not willing to participate in the study.

Results

Table 1: Number of intermediate isolates against beta-lactams, aminoglycosides, and quinolones among culture-positive cases (N=68)

No. of total samples	No. of culture-positive cases	No. of total isolates	No. of intermediate isolates against beta-lactams, aminoglycosides, and quinolones
96	68	73	37

Among 73 isolates from 68 culture-positive cases, 37 isolates showed intermediate susceptibility towards selected antibiotics such as beta-lactams (Amoxicillin/Clavulanic acid, Ceftazidime, Ceftriaxone, and Imipenem), aminoglycosides (Gentamicin) and quinolones (Ciprofloxacin and Levofloxacin). Among 37 intermediate isolates, *P. aeruginosa* was 14 in number.

Table 2: MIC values of ceftazidime among intermediate isolates of *P. aeruginosa* (n=04)

MIC value (µg/ml)	Number of isolates
.016	
.023	
.032	
.047	
.064	
.094	
.125	
.19	
.25	
.38	
.50	
.75	
1.0	01
1.5	
2	01
3	
4	
6	01
8	
12	
16	01
24	
32	
48	
64	
96	
128	
192	
256	

01 intermediate isolates of *P. aeruginosa* had MIC value 1.0 µg/ml, 01 had MIC 2 µg/ml, 01 had 6 µg/ml and 01 had 16 µg/ml.

Table 3: MIC values of ceftriaxone among intermediate isolates of *P. aeruginosa* (n=04)

MIC value (µg/ml)	Number of isolates
.016	
.023	
.032	
.047	
.064	
.094	
.125	
.19	
.25	
.38	
.50	01
.75	
1.0	
1.5	01
2	
3	01
4	
6	
8	
12	01
16	
24	
32	
48	
64	
96	
128	
192	
256	

01 intermediate isolate of *P. aeruginosa* had MIC value 0.50 µg/ml, 01 had 1.5 µg/ml, 01 had 3 µg/ml, and 01 had 12 µg/ml.

Table 4: MIC values of gentamicin among the intermediate isolates of *P. aeruginosa* (n=03)

MIC value (µg/ml)	No. of isolate
.016	
.023	
.032	
.047	
.064	
.094	
.125	
.19	
.25	
.38	
.50	
.75	
1.0	01
1.5	
2	
3	01
4	
6	
8	01
12	
16	
24	
32	
48	
64	
96	
128	
192	
256	

01 intermediate isolate of *P. aeruginosa* had a MIC value of 1 µg/ml, 01 isolate with MIC 3 µg/ml one isolate with MIC 8 µg/ml.

Table 5: MIC values of ciprofloxacin among intermediate isolates of *P. aeruginosa* (n=03)

MIC value (µg/ml)	No. of isolate
.002	
.003	
.004	
.006	
.008	
.012	
.016	
.023	
.032	
.047	
.064	
.094	
.125	
.19	
.25	
.38	
.50	
.75	01
1.0	
1.5	
2	01
3	
4	
6	01
8	
12	
16	
24	
32	

Among 03 intermediate isolates of *P. aeruginosa,* 01 isolates had MIC .75 µg/ml, 01 had a MIC value of 2 µg/ml, and 01 had 6 µg/ml.

Table 6: MIC breakpoints of different antibiotics in terms of susceptibility pattern among the intermediate isolates of *P. aeruginosa* (N=14)

Sl. no	Name of antibiotic	No. of intermediate isolate of *P. aeruginosa*	Susceptible (S)	Intermediate (I)	Resistant (R)
			(≤8 µg/ml)	**(16 µg/ml)**	**(≥32 µg/ml)**
1.	Ceftazidime	04			
			03(75%)	01(25%)	00(00%)
			(≤4 µg/ml)	-	**(≥8 µg/ml)**
2.	Ceftriaxone	04			
			03(75%)	00(00%)	01(25%)
			(≤1 µg/ml)	**(2 µg/ml)**	**(≥4 µg/ml)**
3.	Ciprofloxacin	03			
			01(33.33%)	01(33.33%)	01(33.33%)
			(≤4 µg/ml)	**(8 µg/ml)**	**(≥16 µg/ml)**
4.	Gentamicin	03			
			02(66.66%)	01(33.33%)	00(00%)
Total	**04**	**14**	**09(64.28%)**	**03(21.43%)**	**2(14.28%)**

Regarding MIC breakpoints in terms of susceptibility, out of 14 intermediate isolates of *Pseudomonas aeruginosa,* 09(64.28%) isolates were susceptible, 03(21.43%) intermediate, and 2(14.28%) were resistant to different antibiotics.

Regarding MIC breakpoints in terms of susceptibility, 75% of intermediate isolates of *P. aeruginosa* were susceptible to ceftazidime, and 25 % were intermediate. In addition, 75% of intermediate isolates of *P. aeruginosa* were susceptible to ceftriaxone, and 25 % were resistant. On the other hand, 33.33% of intermediate isolates of *P. aeruginosa* were susceptible to ciprofloxacin, 33.33% were intermediate, and 33.33% were resistant. 66.66% of intermediate isolates of *P. aeruginosa* were susceptible to gentamicin, and 33.33% were intermediate.

Discussion

In this study, isolation, and identification of the causative organisms of CSOM, along with their antimicrobial susceptibility pattern, were made. MIC was determined by an E-test of different drugs (ceftriaxone, ceftazidime, gentamicin, and ciprofloxacin) against intermediate isolates of *P. aeruginosa,* which was a total of 14 in number. MIC values of ceftazidime among four isolates of *P. aeruginosa* ranged from 1-16 µg/ml for ceftazidime. They were 1 µg/ml (for one isolate), 2 µg/ml (for one isolate), 6 µg/ml (for one isolate), and 16 µg/ml (for one isolate) (Table: 2). Data related with this study were very scarce. But according to a study conducted in Chennai, out of 43 strains of *P. aeruginosa,* 03 strains were resistant to ceftazidime having MIC value >16 µg/ml, similar to this study's upper limit[9] Among 04 intermediate isolates of *P. aeruginosa,* MIC values of ceftriaxone ranged from 0.50-12 µg/ml and were 0.50 µg/ml (for one isolate), 1.5 µg/ml (for one isolate), 3 µg/ml (for one isolate) and 12 µg/ml (for one isolate) (Table: 3). In a study in

Italy, it was found that MIC value of ceftriaxone was 8 μg/ml which was similar to this study.[10] Among three intermediate isolates of *P. aeruginosa* MIC value of gentamicin had 1.0 μg/ml (for one isolate), 3 μg/ml (for one isolate), and 8μg/ml (for one isolate) (Table: 4). Satta *et al*. (1988 This study was similar to a study done in Italy (MIC value of Gentamicin 1 μg/ml). [10] Among three intermediate isolates of *P. aeruginosa,* MIC values of ciprofloxacin ranged from 0.75-6 μg/ml and were 0.75 μg/ml (for one isolate), 2 μg/ml (for one isolate) and 6 μg/ml (for one isolate) (Table: 5). This study was similar with a study, where they found the MIC value of Ciprofloxacin against *P. aeruginosa* ranged from 0.06-32 μg/ml.[11] Regarding MIC breakpoints in terms of susceptibility, out of 14 intermediate isolates of *P. aeruginosa*, 9(64.28%) isolates were susceptible, 3(21.43%) were intermediate, and 2(14.28%) isolates were resistant to different antibiotics (Table: 6). According to several studies, higher susceptibility was observed by E-test than disc diffusion method, which was similar with this study.[5,12]

Conclusion

Chronic suppurative otitis media is one of the most common infectious diseases worldwide. In this study, isolation, and identification of the causative organisms of CSOM, along with their antimicrobial susceptibility pattern, were made. In addition, MIC values of ceftriaxone, ceftazidime, gentamicin, and ciprofloxacin were also determined, showing higher susceptibility of these drugs by E-test results than the disc diffusion method.

Conflict of interest: None declared

References

1. Nickson C. Critical care Compendium/Minimum Inhibitory Concentration (MIC). 2017. [Online]. Available at: https://lifeinthefastlane.com/ccc/minimum-inhibitory concentration.
2. Grillon A, Schamm F, Kleinberg M, Jehl F. Comparative Activity of Ciprofloxacin, Levofloxacin, and Moxifloxacin against *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* Assessed by Minimum Inhibitory Concentrations and Time-Kill Studies. Plos One. 2016; 10: 1371.
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, P.A. Clinical and Laboratory Standards Institute. 2017.
4. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of zone diameters. Version 9.0. 2019.
5. Erfani Y, Choobineh H, Safdari R, Rasti A, Alizadeh S. Comparison of E-test and Disk Diffusion Agar in antibiotic Susceptibility of *E. coli* Isolated from patients with urinary tract infections in Shariati Hospital (Iran). Res. J. Biol. Sci. 2011; 3:24-27.
6. Khan AU, Zaman MS. Multiple drug resistance in Urinary Tract Infection patients in Aligarh. Biomed. Res. 2006; 17: 179-181.
7. Rahbar M, Yaghoobi M and Fattahi A. Comparison of different laboratory methods for detection of Methicillin-Resistant *Staphylococcus aureus*. Pak. J. Med. Sci. 2006; 22:442–445.
8. Schumacher Vranken A, Malhotra JJC, Arts and Habibovic P. In vitro antimicrobial susceptibility testing methods: agar dilution to 3D tissue-engineered models. Eur J Clin Microbiol Infect Dis. 2018; 37:187–208.
9. Sankar DP. Evaluation of Multi-Drug Resistant *Pseudomonas aeruginosa* Isolates in Chronic Suppurative Otitis Media. M.D (Thesis). Dr. M.G.R. Medical University, Chennai. 2007.
10. Satta G, Cornaglia G, Foddis G, Pompei R. Evaluation of Ceftriaxone and Other Antibiotics against Escherichia coli, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* under In Vitro Conditions Simulating Those of Serious Infections. Antimicrobial Agents and Chemotherapy. 1988; 32:552-560.
11. Ikeda K, Misawa S, Kusunoki T. Comparative bactericidal activity of four fluoroquinolones against *Pseudomonas aeruginosa* isolated from chronic suppurative otitis media. BMC Ear Nose Throat Disord. 2015; 15: 5.
12. Khalili H, Soltani R, Negahban S, Abdollahi A, Gholami K. Reliability of Disk Diffusion Test Results for the Antimicrobial Susceptibility Testing of Nosocomial Gram-positive Microorganisms: Is E-test Method Better? Iran J Pharm Res. Spring. 2012; 11(2): 559–563.

All correspondence to
Dr. Mst. Romena Khatun
Assistant Professor (C.C)
Department of Microbiology
Rajshahi Medical College, Rajshahi.
E-mail: romenak6@gmail.com