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Original Research Article

Determination of MIC of Different Antibiotics against Intermediate Isolates of *S. aureus* at a Tertiary Care Hospital by E-test

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Abstract

Background: The E-test involves using a predefined antibiotic gradient on a strip, which is then placed on an agar plate containing the bacterial culture. The point at which the antibiotic concentration on the strip causes inhibition of bacterial growth is taken as the MIC. This method allows for the rapid and accurate determination of the MIC of antibiotics against specific bacterial strains and can aid in selecting appropriate antibiotics for treatment. ***Objectives:*** The aim of this study was also to include a comparison of the results with those of previous studies and an evaluation of the study's limitations. ***Method:*** A cross-sectional microbiological study was conducted at the Department of Microbiology, and the Department of ENT Rajshahi Medical College, Bangladesh, from January to December 2019. This section will provide a detailed description of the methods used in the study, including the study design, sampling methods, and procedures for collecting and analyzing the data. The methods section will also explain the E-test method used to determine MIC, including the procedure for performing the test and the criteria for interpreting the results. ***Results:*** A total of 96 samples, among 73 isolates from 68 culture-positive cases, 37 isolates showed intermediate susceptibility towards selected antibiotics such as beta-lactams, aminoglycosides and quinolones by disc diffusion method. Regarding MIC breakpoint in terms of susceptibility, out of 21 intermediate isolates of *S. aureus*, 16(76.2%) were susceptible, 01(4.8%) was intermediate, and 04(19%) were resistant to different antibiotics by E-test. ***Conclusion:*** In general, the conclusion of such an article would likely summarize the study's findings, such as the MIC values for the different antibiotics tested and how they compare to established MIC breakpoints, and any significant observations or trends noted. It may also discuss the clinical relevance of the results and provide recommendations for future research.

Keywords: *S. aureus*, antimicrobial susceptibility.

INTRODUCTION

Antimicrobial drug resistance is a matter of concern worldwide. Antimicrobial susceptibility test (AST) can be done by determining MIC. E-test is a recent method for determination of MIC. Disc diffusion agar is a standard qualitative method for determining antibiotic susceptibility. Because of its possible limitations, MIC-based methods like E-test have been developed [1]. Antimicrobial susceptibility testing (AST) by disc diffusion method is functional, but it is a qualitative test. On the other hand, determining the minimum inhibitory concentration (MIC) of a particular drug against a particular bacterium is more effective for treatment because it is both a qualitative and a quantitative test [2].

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism following overnight incubation, usually reported as mg/L [3]. The lower the MIC, the better the bactericidal activity in the CFU log decreases [4]. According to the Clinical and Laboratory Standards Institute, the results of AST were categorized as susceptible (S), intermediate (I), and resistant (R) based on MIC breakpoints [5]. But according to European Committee on Antimicrobial

Susceptibility Testing, the intermediate (I) category is termed recently as 'I' - susceptible, increased exposure and is defined as a microorganism is categorized as susceptible. Increased exposure is 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 infection. Usually, many clinicians consider the intermediate (I) category drug as resistant (R) 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 [1].

In our country, the commonly used drugs against Gram-positive and Gram-negative bacteria are usually beta-lactams, aminoglycosides, quinolones, etc. Determination of 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 quantify microorganisms' antimicrobial susceptibility directly. MIC-determining methods like E-test provide quantitative measurement of antimicrobial susceptibility. Because of their cost and limited availability in developing countries. Still their application is not as frequent as the disc diffusion method [6,7]. But, it has an extensive range of over 100 antimicrobial references that can be classified into 4 categories such as antibiotics, antifungal, anti-mycobacterial, and resistance phenotype testing.

General objective:

- Determination of minimum inhibitory concentration of deferent antibiotics against intermediate isolates of *S. aureus* by E-test.

MATERIALS AND METHODS

A cross-sectional microbiological study (N=96) was conducted at the Department of Microbiology, Rajshahi Medical College, Bangladesh and Department of ENT, Rajshahi Medical College Hospital, Rajshahi from January to December 2019.

Inclusion Criteria

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

Exclusion Criteria

- Acute suppurative otitis media.
- Chronic otitis media with effusion.
- Patients are not willing to participate in the study.

Data Analysis:

All relevant information and laboratory findings were recorded in the datasheet. The data were analyzed using SPSS software, programmed version 23.0. A descriptive analytic technique was applied, involving frequency distribution, computation of percentage, mean, standard deviation, etc. After data analysis, results were found according to the objectives. Study results were presented in tables, charts, graphs, and descriptions of the key findings.

Ethical Consideration

Clearance from the Ethical Review Committee of Rajshahi Medical College was taken to carry out this study. Clearance was taken from the proper authority of Rajshahi Medical College Hospital and respective departments. Informed consent was taken from the study subject after proper information regarding the study design, objectives of the study, methodology, advantages, and disadvantages of enrollment, etc., was given to the individual subject.

RESULTS

A total of 96 specimens (aural swabs) were collected from clinically suspected patients of CSOM. Regarding MIC breakpoints in terms of susceptibility, 100% intermediate isolates of *S. aureus* were susceptible to Levofloxacin. 80% of intermediate isolates of *S. aureus* were susceptible to Ceftriaxone, and 20% were resistant. 80% of intermediate isolates of *S. aureus* were susceptible to Amoxicillin/Clavulanic acid, and 20% were resistant. 60% of intermediate isolates of *S. aureus* were susceptible to Ciprofloxacin, 10% were intermediate, and 10% were resistant.

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

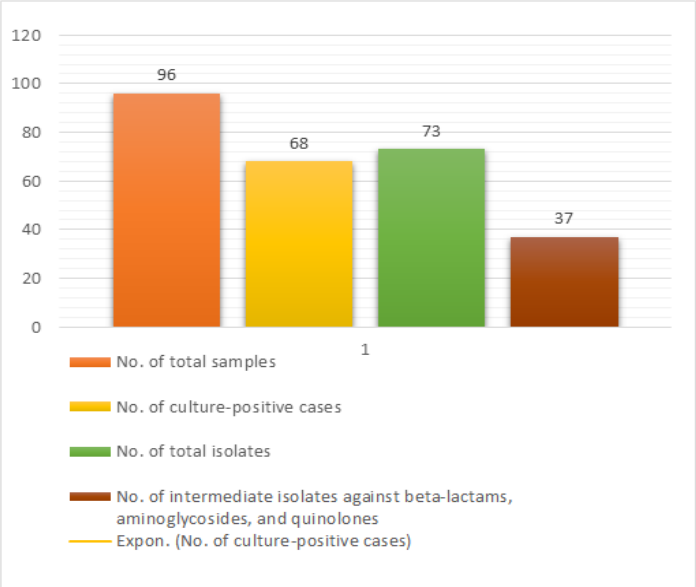


Figure-1: Number of intermediate isolates against beta-lactams, aminoglycosides, and quinolones among culture-positive cases CSOM

Among 73 isolates 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) by disc diffusion method.

Table 2: MIC values of Amoxicillin/Clavulanic acid among intermediate isolates of *S. aureus* (n=10)

Sl. No	MIC value (µg/ml)	Number of isolates
01	.016	
02	.023	
03	.032	
04	.047	
05	.064	
06	.094	
07	.125	
08	.19	
09	.25	
10	.38	
11	.50	
12	.75	
13	1.0	04
14	1.5	02
15	2	01
16	3	01
17	4	
18	6	
19	8	01
20	12	01
21	16	
22	24	
23	32	
24	48	
25	64	
26	96	
27	128	
28	192	
29	256	

Among 21 intermediate isolates of *S. aureus*, 04 had a MIC value of 1.0 µg/ml, followed by 02 isolates had 1.5 µg/ml, 01 had 2 µg/ml, 01 had 3 µg/ml, 01 had 8 µg/ml, and 01 had 12 µg/ml.

Table 3: MIC values of Ceftriaxone among intermediate isolates of *S. aureus* (n=05)

Sl. No	MIC value (µg/ml)	Number of isolates
01	.016	
02	.023	
03	.032	
04	.047	
05	.064	
06	.094	
07	.125	
08	.19	
09	.25	
10	.38	
11	.50	
12	.75	
13	1.0	01
14	1.5	01
15	2	02
16	3	
17	4	
18	6	
19	8	01
20	12	
21	16	
22	24	
23	32	
24	48	
25	64	
26	96	
27	128	
28	192	
29	256	

02 intermediate isolates of *S. aureus* had a MIC value of 2.0 µg/ml, followed by 01 isolate had 1 µg/ml, 01 had 1.5 µg/ml, and 1 had 8 µg/ml.

Table 4: MIC values of Ciprofloxacin among intermediate isolates of *S. aureus* (n=05)

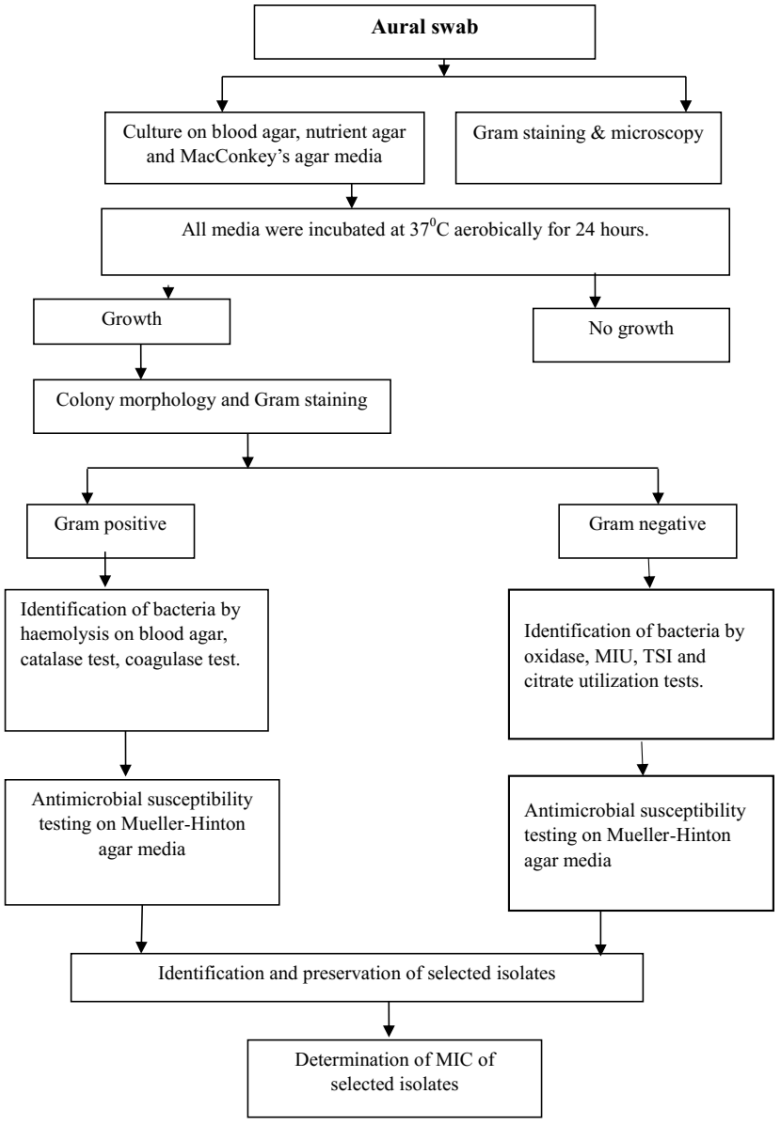
Sl. No	MIC value (µg/ml)	Number of isolates
01	.002	
02	.003	
03	.004	
04	.006	
05	.008	
06	.012	
07	.016	
08	.023	
09	.032	
10	.047	
11	.064	
12	.094	
13	.125	01
14	.19	
15	.25	
16	.38	01
17	.50	
18	.75	01
19	1.0	
20	1.5	
21	2	01
22	3	
23	4	
24	6	01
25	8	
26	12	
27	16	
28	24	
29	32	

Among 05 intermediate isolates of *S. aureus*, 01 had a MIC value of 0.125 µg/ml, 01 had 0.38 µg/ml, 01 had a MIC value of 0.75 µg/ml, 01 had a MIC value of 2 µg/ml, and 01 had MIC 6 µg/ml.

Table 5: MIC breakpoints of different antibiotics in terms of susceptibility pattern among intermediate isolates of *S. aureus* (N=21)

Sl. no	Name of antibiotic	No. of an intermediate isolate of *S. aureus*	Susceptible (S)	Intermediate (I)	Resistant (R)
1.	Amoxicillin/ Clavulanic acid	10	**(≤4 µg/ml)** 08(80%)	- 00 (00%)	**(≥ 8µg/ml)** 02(20%)
2.	Ceftriaxone	05	**(≤4 µg/ml)** 04(80%)	- 00(00%)	**(≥8 µg/ml)** 01(20%)
3.	Ciprofloxacin	05	**(≤1 µg/ml)** 03(60%)	**(2 µg/ml)** 01(20%)	**(≥4 µg/ml)** 01(20%)
4.	Levofloxacin	01	**(≤4 µg/ml)** 01(100%)	**(8 µg/ml)** 00(00%)	**(≥16 µg/ml)** 00(00%)
Total	**04**	**21**	**16(76.2%)**	**01(4.8%)**	**04(19%)**

Shows MIC breakpoints of different antibiotics in terms of susceptibility pattern among the intermediate isolates of *S. aureus*. Regarding MIC breakpoints in terms of susceptibility, out of 21 intermediate isolates of *S. aureus*, 16(76.2%) were susceptible, 01(4.8%) was intermediate, and 04(19%) were resistant to different antibiotics.



Flow chart (Identification of causative bacteria):

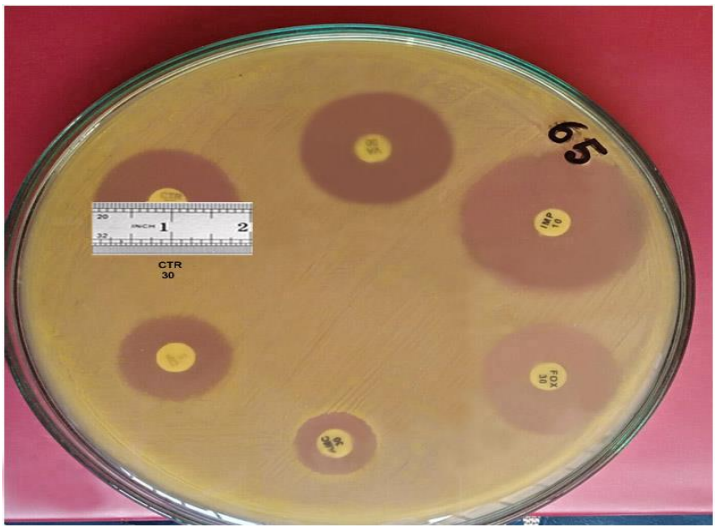


Figure-2: Antibiogram of *S. aureus* on Mueller-Hinton agar (zone of inhibition of Ceftriaxone representing 'Intermediate') (By disc diffusion method)

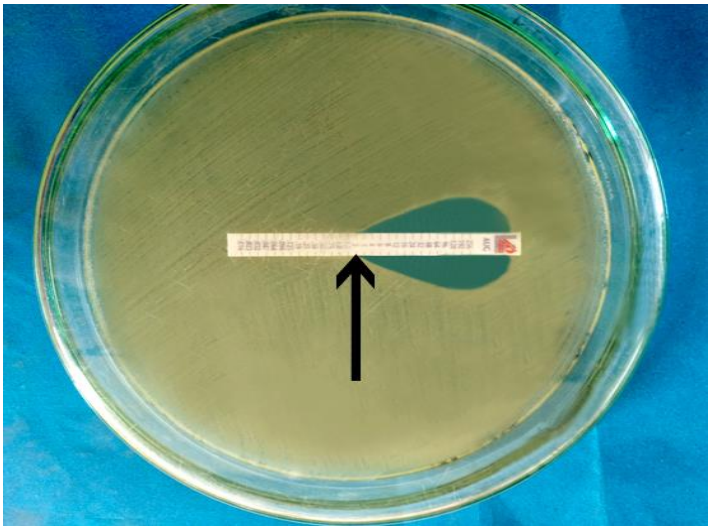


Figure-3: E-test of *S. aureus* showing MIC value of Amoxicillin/Clavulanic acid 2 µg/ml

Figure-4: E-test of *S. aureus* showing MIC value of Ceftriaxone 1.5 µg/ml

DISCUSSION

In this present study, total 96 specimens were collected from the of Department of ENT, Rajshahi Medical College Hospital, Rajshahi. Out of 73 isolates, 37 were categorized as intermediate towards beta-lactams (Amoxicillin/Clavulanic acid, Ceftriaxone, Ceftazidime, and Imipenem), aminoglycosides (Gentamicin) and quinolones (Ciprofloxacin and Levofloxacin) by disc diffusion method (Table 1). Practically intermediate isolates are thought as resistant and are not used for treatment purpose. But according to, an intermediate category is renamed as 'I' - susceptible, increased exposure. Thereby, by determining the MIC of the intermediate isolates, the exact dose of that particular drug can be determined, and many common and cost-effective drugs can be used effectively.

In this study, 21 isolates of *S. aureus* were intermediate to different antibiotics. Among them, 10 isolates were intermediate to Amoxicillin/Clavulanic acid having MIC values ranging from 1-12 µg/ml and were 1.0 µg/ml (for 04 isolates) followed by 1.5 µg/ml (for 02 isolates), 2 µg/ml (for 01 isolates), 3 µg/ml (for 01 isolates), 8 µg/ml (for 01 isolates) and 12 µg/ml (for 01 isolates) (Table 2). Very few data were available regarding this study. According to [8], in America, MIC values ranged from 1-16 µg/ml, which was more or less similar to this study. Dissimilarity was shown in Poland, where MIC values ranged from 0.03-2 µg/ml. MIC values of Ceftriaxone among 05 isolates of *S. aureus* were 2.0 µg/ml (for 02 isolates) followed by 1 µg/ml (for 01 isolate), 1.5 µg/ml (for 01 isolates) and 8 µg/ml (for 01 isolates) which was ranging from 1-8 µg/ml (Table 3) [9]. MIC values ranged from 4 to >64 µg/ml, which was dissimilar from this study [10]. This may be due to geographical variation, including susceptible and resistant isolates, etc.

Out of total 05 intermediate isolates of *S. aureus*, MIC values of Ciprofloxacin were 0.125 µg/ml (for 01 isolate), 0.38 µg/ml (for 01 isolate), 0.75 µg/ml (for 01 isolate), 2 µg/ml (for 01 isolates) and 6 µg/ml (for 01 isolate) and was ranging from 0.125-6 µg/ml where MIC values of Ciprofloxacin were ranging from 0.03-16 µg/ml and 0.5-16 µg/ml which was also quite similar with this study. According to Seral *et al.*, [4], in Belgium MIC value of Ciprofloxacin was 0.125 µg/ml and was similar to the lower limit of this study. The MIC value of Levofloxacin of 01 intermediate isolate was 1.5 which was dissimilar with the study. This dissimilarity may be due to geographical variation and lack of adequate relevant data. Regarding MIC breakpoints in terms of susceptibility, out of 21 intermediate isolates of *S. aureus* 16(76.2%) isolates were susceptible, 01(4.8%) were intermediate and 04(19%) isolates were resistant to different antibiotics. By E-test we found 76.2% intermediate isolates were susceptible, 4.8% were intermediate and 19% were resistant to different antibiotics. An interesting feature was observed by Gautam *et al.*, [11] and various other studies conducted worldwide and that was the lack of correlation between the results of disc diffusion and MIC methods. These variations could be due to fewer isolates being tested with MIC and may be due to random selection of the isolates. performed E-test of resistant and intermediate isolates (determined by disc diffusion method) and found around 11-48% susceptible to different antibiotics by E-test.

In this study, isolation, and identification of the causative organisms of CSOM, along with their antimicrobial susceptibility pattern, was done. MIC values of Amoxicillin/Clavulanic acid, Ceftriaxone, Ceftazidime, Imipenem, Gentamicin, Ciprofloxacin, and Levofloxacin were also determined to show higher susceptibility by E-test results than disc diffusion method. So, clinicians should follow the antimicrobial susceptibility test results to choose the appropriate drugs and doses to treat these types of chronic infections to reduce morbidity and mortality.

CONCLUSION

Gram-positive and Gram-negative aerobic bacteria were associated with cases of CSOM, and many were resistant to different antibiotics. In this study, most intermediate isolates (observed by disc diffusion method) were susceptible to the selected drugs (determined by E-test). This section will present the study's findings, including data on the MIC values for different antibiotics against intermediate strains from auricular swab samples.

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Conflict of Interest: None.

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