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Evaluation of Penicillins minimum inhibitory concentration against clinically isolated *Streptococcus pyogenes*

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Abstract*---*Streptococcus specie is the crucial cause of pharyngitis and tonsillitis in human particularly in youngsters and is the main organisms transmitted from the milk to human. *Streptococcus pyogenes* cause diseases worldwide, thus increases huge burden to national health care systems. The current study was take on to determine antibiotic resistance pattern of *S. pyogenes* against penicillin group of antibiotics by disc diffusion method. Furthermore, minimum inhibitory concentration of penicillin group of antibiotics were assisted against *S. pyogenes* isolates. Three hundred samples including sore throat, skin lesion and surgical wounds were collected from different patients. The clinical specimens were cultured on blood agar media to observe hemolysis and were identified with Bacitracin sensitivity test. To evaluate the resistance pattern of Penicillin's antibiotic sensitivity test was done. Different antibiotics used were Penicillin G (10 U), Ampicillin (10 mcg), Amoxicillin (25 mcg), and Piperacillin (100 mcg) respectively. Minimum inhibitory concentration was checked against *S. pyogenes* by using four antibiotics i.e. Penicillin G, Piperacillin, Ampicillin and Amoxicillin. A total of 17 isolates showed positive growth for *S. pyogenes* i.e. from throat specimens the number of positive isolates were 10, surgical wounds showed 05 and skin pus were positive for 02 isolates. Maximum number of isolates (14) showed resistance against Ampicillin (82.35%) followed by Penicillin G (76.47%) and Amoxicillin (41.17%) while minimum resistance was observed against Piperacillin (29.41%). Similarly, the maximum rate of susceptibility was observed against Piperacillin (41.17%) followed by Amoxicillin (29.41%). The maximum value of MIC against Penicillin G, Ampicillin observed for all the isolates was 06 ppm (0.006 mg/mL). *S. pyogenes* showed noticeable resistance to Penicillin, Amoxicillin and Piperacillin. Highest MIC value was observed against Penicillin and Ampicillin 06 ppm (0.006 mg/mL) while displayed Amoxicillin 4ppm (0.004 mg/mL). At local level continuing surveillance of bacterial antimicrobial sensitivity tests are necessary to decrease the emergence and extent of resistant bacterial isolates.

Keywords*---*Streptococcus pyogenes, pharyngitis, hemolysis, resistant, minimum inhibitory concentration.

Introduction

Infections triggered by resistant bacteria frequently fail to react to the normal treatment ensuing in sustained sickness, advanced health care expenses and a larger threat of expiry. The effectiveness of treatment thus reduces in patients due to antimicrobial resistance (Ratemo, 2014). A worldwide pattern of expanding antimicrobial resistance is all around reported in the literature. Strong proof

supports a relationship between anti-infection use and resistance in doctor's facilities. By complexity, the relationship between anti-microbial utilization and resistance has been harder to build up for the outpatient setting, although a few information recommend an immediate connection for streptococcal contaminations (Rizwan *et al.*, 2016).

Streptococcus pyogenes can be solely human agent. It belongs to the serological A amid the streptococci (GAS). GAS can be main rationalization for diseases so, upsurges vast liability to national health care systems. It causes number of infections in humans extending from subtle skin and tract infections to severe settings like blood dyspraxia, pneumonia, necrotizing fasciitis and modern syndrome. *S.pyogenes* is the utmost joint organism rationalization for raw throat that's extra common in settings of impoverishment (Hameed *et al.,* 2022).

GAS is that the most typical microorganism reason for inflammatory disease in kids and adolescents with a peak incidence in winter and early spring. GAS inflammatory disease is additionally additional common in school-aged kids or in those with a right away respect to school-aged kids. A recent meta-analysis showed that the prevalence GAS inflammatory disease in those but eighteen years previous United Nations agency skill to a patient center for treatment for a pharyngitis was thirty seventh, and for kids younger than five, it was 24%.In adults, however, GAS inflammatory disease can usually occur before the age of forty and decline steady subsequently (Marais *et al.,* 2019)

Antibiotic resistance pattern of this organism has been neutering in recent years, it's mostly as of unfit usage of broad spectrum antibiotics. The frequency of resistance of GAS to varied antibiotics is increasing worldwide. Now, penicillin is that the drug of alternative for GAS sore throat and antibiotic drug resistance for GAS has not been rumored. Nevertheless, the prevalence of antibiotic resistance among GAS is increasing day by day (Yasir *et al.,* 2019).

Penicillin and its derivatives stand the medication of selection for streptococcic pharyngotonsillitis as *S. pyogenes* ruins uniformly at risk of them. Macrolides and Lincosamides measure the vital conduct for *S. pyogenes* disease in patients those are sensitive to beta-lactam anti-microbial drugs (Zhang *et al.,* 2022). Patients with serious delicate tissue contaminations e.g. necrotizing fasciitis, Clindamycin is used for their treatment. Solidity to macrolides and Lincosamides isn't seldom found in *S. pyogenes*, once it's known, Fluoroquinolones (FQs) square measure a useful possibility (Usman *et al.,* 2020). In patients wherever, allergic reaction or non-compliance foils the employment of penicillin's, macrolides square measure used. Macrolide resistance (5 - 50%) and therapeutic failures have additionally been determined to get on the increase worldwide, thanks to their exaggerated prescription (Capoor *et al.,* 2018)

Penicillin-resistant and penicillin-tolerant mutants of *S. pyogenes* were in-vitro isolated after the usage by ethyl-methane-sulfonate. These strains, swift low-affinity PBPs, undisputable a 32-fold rise in penicillin G MICs (from 0.006 to 0.2 µg/ml), on the other hand unconcealed severe physiological faults to scale back the expansion rates and gross morphological abnormalities. These conclusion

acclaim that as clinical isolates the strains show less chance to expand, whereas, recognition spotted in such mutants doesn't have clinical name (Cattoir, 2016).

Natural resistance to penicillin's arises in organisms that furthermore, lack peptidoglycan semipermeable membrane or has cell walls that are immune to the medication. No inheritable resistance to penicillin's by inclusion transfer has develop a significant clinical downside, as a result of Associate in Nursing organism might come back to be immune to many antibiotics at identical time thanks to attainment of a inclusion that codes resistance to multiple agents (Kannan *et al.,* 2014). The 2 core mechanisms of macrolide resistance in *Streptococci* are: target website alteration and macrolide flow system. The primary is adept by multiplicity of enzymes rRNA methylases that methylate in rear purine deposit of the 23S rRNA V domain. It stimulates a modification that declines the binding of Lincosamides, macrolides and streptogramins B to ribosomes, and offer co-resistance to those anti-infection agents i.e. the MLSB composition (Rubio-López *et al.*, 2012).

The minimum inhibitory concentration is well-defined as the lowest concentration of the anti-microbial agent that is necessary to stop the growth of a microbes in different conditions. This method contributes a quantitative measure of bacterial susceptibility. The lowest concentration of an anti-microbial agent that evades visible growth of a micro-organism in an agar or broth dilution susceptibility test (Andrews, 2001)

Materials and Methods

Sample Collection

The study was conducted at Microbiology Laboratory, Abasyn University Peshawar. About 300 samples including sore throat, skin lesion and surgical wounds were collected from different patients by using sterile swabs and syringes from three major hospitals (LRH, HMC and KTH) of Peshawar. For the isolation and identification of *Streptococcus pyogenes* isolates samples were collected.

Sample Processing

Samples were transferred to Microbiology Laboratory, Abasyn University Peshawar immediately after collection and then streaked on Blood Agar and Nutrient Agar media plates. The plates were incubated for 24 hours at 37°C. Cultures obtained on each plate were further processed for species identification by Gram staining and biochemical tests. Different biochemical tests done to identify bacterial species from Gram-positive isolates were Catalase test, oxidase test, and bacitracin sensitivity test. Then antibiotic sensitivity profiles and determination of MIC was performed.

Antimicrobial sensitivity test

The disc diffusion technique is use for Antibiotic sensitivity test. On nutrient broth, micro-organism were inoculated and then spread on Muller-Hinton agar plates. By the help of forceps, different antibiotics discs were set on the

inoculated plates. Then, plates were incubated for twenty-four hours at 37°C. Zone of inhibition was calculated in mm (Reller, 2009). Test was performed on Muller-Hinton agar (MHA) by disc-diffusion method contrary to the antibiotics i.e. Penicillin G (10 U=10 µg), Ampicillin (10 mcg), Amoxicillin (25mcg), and Piperacillin (100 mcg) on completely different isolates. For incubation, plates were incubated at 37°C for twenty-four hours after the zone of inhibition was measured in mm. In writing the results, S illustrated sensitivity of the organism to the antibiotic though, resistance to any antibiotic was represented by R (Wayne, 2005).

Determination of MIC by Agar Dilution Method

Agar dilution method was used for the determination of minimum inhibitory concentration. (NCCLS, 2014). The isolates show resistance against different antibiotics were then exposed to the determination of MIC by agar dilution method on Muller Hinton Agar with 5% sheep blood in the environment of 5% CO_2 following the CLSI break points (CLSI, 2016).

Five gram of each antibiotic was taken and dissolved in the required solvent and the stock solution was made according to the following formula Molarity = Molecular weight of the antibiotic/Dissolved in 1000 ml of solvent. Five sterilized test tubes were taken and were labeled as 1 ppm up to 10 ppm. Ten different dilution of antibiotics were prepared as 1 ppm up to 10 ppm of 5 ml each by using formula i.e. $P_1V_1=P_2V_2$. 200 ml nutrient agar was made. Then eleven flasks were taken and labelled as 1 ppm up to 10 ppm and control. Twenty-five ml media was added in each flask from 200 ml media whereas the required dilutions of each antibiotic was then added in the flask. Eleven petri plates were taken and labeled as 1 ppm up to 10 ppm and control. Media containing antibiotics were poured in the plates and *S. pyogenes* were spread over them. The plates were placed for 24 hours at 37°C. Then the growth was perceived around the plates on the next day.

Results

In the present study, total three hundred samples were screened out in totally different clinical specimens as shown in Table 1. Out of the tested samples, 17 *S. pyogenes* spp. were isolated. *S. pyogenes* were β-hemolytic on blood agar plates and produced a clear zone around the colony because of complete hemolysis of RBC's. The isolates were Gram positive cocci and were in chain form under microscope. Catalase and oxidase tests were both negative for the species and they were found sensitive to bacitracin antibiotic.

For Amoxicillin, the SP28 (12 mm), SP49 (13 mm), SP55 (10 mm), SP79 (08 mm), SP92 (13 mm), SP112 (11 mm), SP149 (09 mm) were found resistant according to CLSI (2016) while the susceptible isolates were SP172 (20 mm), SP191 (25 mm), SP228 (18 mm), SP237 (23 mm), and SP271 (18 mm). The intermediate isolates observed for Amoxicillin were SP09 (14 mm), SP157 (16 mm), SP209 (17 mm), SP256 (14 mm) and SP266 (17 mm).

Similarly, for Ampicillin SP09 (13 mm), SP28 (12 mm), SP49 (17 mm), SP55 (14 mm), SP79 (16 mm), SP92 (12 mm), SP112 (15 mm) were found resistant while the susceptible isolates were SP209 (25 mm) and SP228 (24 mm) and one isolate SP149 (21 mm) showed intermediate susceptibility towards this antibiotic.

Similarly, for Piperacillin SP28 (15 mm), SP92 (17 mm), SP149 (09 mm), SP172 (13 mm), SP271 (17 mm) were found resistant while the susceptible isolates observed were SP79, SP112, SP157, SP191, SP228, and SP256 showing 26 mm, 25 mm, 29 mm, 21 mm and 25 mm zone of inhibition. The intermediate isolates were SP09, SP55, SP209, SP237 and SP266 showing 20 mm, 20 mm, 19 mm, 18 mm and 19 mm ZI respectively.

High antibiotic resistance was observed in *S. pyogenes* isolates for Penicillin G. The resistant isolates were SP09 (20 mm), SP28 (22 mm), SP49 (19 mm), SP112 (24 mm), SP149 (18 mm), SP157 (17 mm), SP172 (20 mm), SP191 (16 mm), SP209 (26 mm), SP228 (20 mm), SP256 (15 mm), SP266 (13 mm) and SP271 (14 mm). Intermediate isolates were SP55 (29 mm), SP79 (32 mm) and SP237 (27 mm) while one isolate SP92 (39 mm) was found susceptible to the antibiotic as shown in Table 3.

Table 1: Percentage occurrences of *S. pyogenes* from different clinical specimens.

S. No	Sources	Sample Collected	Positive Sample	Percentage
1	Throat	120	10 (M=6, F=4)	8.3%
2	Surgical Wounds	50	05 (M=3, F=2)	10%
3	Skin Pus	130	02 (M=2, F=0)	1.5%

Key: M= Male, F= Female.

Table 2: Zone Diameter Interpretive Standards for *Streptococcus pyogenes* according to CLSI 2016.

S.No.	Antibiotics	Zone Diameter Interpretive Criteria (nearest whole mm)		
		S	I	R
1.	Amoxicillin	≥18	14-17	≤13
2	Ampicillin	≥ 24	18-24	≤17
3.	Piperacillin	≥21	18-20	≤17
4.	Penicillin G	≥ 24	18-24	≤17
5.	Erythromycin	≥ 21	16-20	≤15
6.	Telithromycin	≥ 19	16-18	≤15
7.	Azithromycin	≥18	14-17	≤13
8.	Clarithromycin	≥21	17-20	≤16

Key: S= Susceptible, I= Intermediate, R= Resistant.

Table 3: Antibiotic susceptibility pattern of *S. pyogenes* to penicillin group of antibiotics

S. No	Sample	Zone of inhibition against antibiotics (mm)			
		Amoxicillin	Ampicillin	Piperacillin	Penicillin G
1	SP09	14	13	20	20
2	SP28	12	12	25	19
3	SP49	13	17	15	22
4	SP55	10	14	20	29
5	SP79	08	16	26	32
6	SP92	13	12	17	39
7	SP112	11	15	27	24
8	SP149	09	21	09	18
9	SP157	16	13	29	17
10	SP172	20	11	13	20
11	SP191	25	17	26	16
12	SP209	16	25	19	26
13	SP228	18	24	21	20
14	SP237	23	13	19	27
15	SP256	17	13	25	15
16	SP266	14	15	18	13
17	SP271	18	14	17	14

Minimum Inhibitory Concentration

All the isolates showed positive growth while using Penicillin G antibiotic at the concentration of 1 mg/ml, 2 mg/ml and 3 mg/ml respectively. On the other hand, at concentration 4 mg/ml and 5 mg/ml isolates encoded as SP49, SP191, SP237, SP 266, SP09, SP28 , SP49, SP55, SP92, SP112, SP157, SP191, SP209, SP237, SP256, SP266 and SP271 showed no growth while the remaining isolates displayed sufficient growth at concentration of a 4 mg/ml and 5 mg/ml of Penicillin G. Furthermore, at concentration of 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml and 10 mg/ml no growth was observed while the control showed growth of all the *S. pyogenes* isolates.

Table 4: Minimum inhibitory concentration (MIC) of Penicillin G against *S. pyogenes* isolates at different concentrations (ppm).

S. No	Sample	MIC of Penicillin G against Different Isolates at Different ppm (mg/mL)										Control
		1 ppm	2 ppm	3 ppm	4 ppm	5 ppm	6 ppm	7 ppm	8 ppm	9 ppm	10 ppm	
1	SP09	+	+	+	+	-	-	-	-	-	-	+
2	SP28	+	+	+	+	-	-	-	-	-	-	+
3	SP49	+	+	+	-	-	-	-	-	-	-	+
4	SP55	+	+	+	+	-	-	-	-	-	-	+
5	SP79	+	+	+	+	+	-	-	-	-	-	+
6	SP92	+	+	+	+	-	-	-	-	-	-	+
7	SP112	+	+	+	+	-	-	-	-	-	-	+

8	SP149	+	+	+	+	+	-	-	-	-	-	+
9	SP157	+	+	+	+	-	-	-	-	-	-	+
10	SP172	+	+	+	+	+	-	-	-	-	-	+
11	SP191	+	+	+	-	-	-	-	-	-	-	+
12	SP209	+	+	+	+	-	-	-	-	-	-	+
13	SP228	+	+	+	+	+	-	-	-	-	-	+
14	SP237	+	+	+	-	-	-	-	-	-	-	+
15	SP256	+	+	+	+	-	-	-	-	-	-	+
16	SP266	+	+	+	-	-	-	-	-	-	-	+
17	SP271	+	+	+	+	-	-	-	-	-	-	+

Key: '+' = Growth observed, '-'= No growth, '1 ppm'= 0.001 mg/ml.

In case of Ampicillin, all the isolates showed positive growth at the concentration of 1 mg/ml, 2 mg/ml and 3 mg/ml. On the other hand, at the concentration of 4 mg/ml and 5 mg/ml isolates coded as SP09, SP28, SP49, SP55, SP79, SP92, SP112, SP149, SP157, SP172, SP191, SP228, SP256, SP266 and SP271 showed no growth whereas the remaining isolates presented sufficient growth at the concentration of 4 mg/ml and 5 mg/ml of Ampicillin. Moreover, no growth was observed against any isolate at concentration of 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml and 10 mg/ml although the control indicated growth of all *S. pyogenes* isolates.

In case of Piperacillin, all the isolates showed positive growth at the concentration of 1 mg/ml, 2 mg/ml and 3 mg/ml. On the other hand, at the concentration of 4 mg/ml and 5 mg/ml isolates coded as SP09, SP28, SP49, SP55, SP79, SP92, SP112, SP149, SP157, SP172, SP191, SP228, SP256, SP266 and SP271 showed no growth whereas the remaining isolates presented sufficient growth at the concentration of 4 mg/ml and 5 mg/ml of Piperacillin. Moreover, no growth was observed against any isolate at concentration of 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml and 10 mg/ml although the control indicated growth of all *S. pyogenes* isolates.

Amoxicillin was the most effective antibiotic that inhibit the growth of *S. pyogenes* isolates at low concentrations. At concentration 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0. 6 mg/ml, 0.5 mg/ml isolates encoded as SP09, SP28, SP49, SP55, SP79, SP 92, SP112, SP149, SP157, SP172, SP191, SP209, SP228, SP237, SP256, SP256, SP266 and SP271 showed no growth while the control showed growth of all the *S. pyogenes* isolates. All the isolates showed positive growth while using Amoxicillin antibiotic at the concentration of 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml and 0.1 mg/ml respectively as shown in Table 5-10.

Table 5: Minimum inhibitory concentration (MIC) of Ampicillin against *S. pyogenes* isolates at different concentration (ppm).

S.NO	Sample	MIC of Ampicillin against different isolates at different ppm (mg/ mL)										Control
		1 ppm	2 ppm	3 ppm	4 ppm	5 ppm	6 ppm	7 ppm	8 ppm	9 ppm	10 ppm	
1	SP09	+	+	+	+	-	-	-	-	-	-	+
2	SP28	+	+	+	+	-	-	-	-	-	-	+

3	SP49	+	+	+	-	-	-	-	-	-	-	+
4	SP55	+	+	+	+	-	-	-	-	-	-	+
5	SP79	+	+	+	+	-	-	-	-	-	-	+
6	SP92	+	+	+	-	-	-	-	-	-	-	+
7	SP112	+	+	+	+	-	-	-	-	-	-	+
8	SP149	+	+	+	-	-	-	-	-	-	-	+
9	SP157	+	+	+	+	-	-	-	-	-	-	+
10	SP172	+	+	+	-	-	-	-	-	-	-	+
11	SP191	+	+	+	-	-	-	-	-	-	-	+
12	SP209	+	+	+	+	+	-	-	-	-	-	+
13	SP228	+	+	+	-	-	-	-	-	-	-	+
14	SP237	+	+	+	+	+	-	-	-	-	-	+
15	SP256	+	+	+	-	-	-	-	-	-	-	+
16	SP266	+	+	+	-	-	-	-	-	-	-	+
17	SP271	+	+	+	+	-	-	-	-	-	-	+

Key: '+' = Growth observed, '-'= No growth, '1 ppm'= 0.001 mg/ml.

Table 6: Minimum inhibitory concentration (MIC) of Piperacillin against *S. pyogenes* isolates at different concentration (ppm).

S.NO	Sample	MIC of Piperacillin against different isolates at different ppm (mg/ mL)										Control
		1 ppm	2 ppm	3 ppm	4 ppm	5 ppm	6 ppm	7 ppm	8 ppm	9 ppm	10 ppm	
1	SP09	+	+	+	+	-	-	-	-	-	-	+
2	SP28	+	+	+	+	-	-	-	-	-	-	+
3	SP49	+	+	+	-	-	-	-	-	-	-	+
4	SP55	+	+	+	+	-	-	-	-	-	-	+
5	SP79	+	+	+	+	-	-	-	-	-	-	+
6	SP92	+	+	+	-	-	-	-	-	-	-	+
7	SP112	+	+	+	+	-	-	-	-	-	-	+
8	SP149	+	+	+	-	-	-	-	-	-	-	+
9	SP157	+	+	+	+	-	-	-	-	-	-	+
10	SP172	+	+	+	-	-	-	-	-	-	-	+
11	SP191	+	+	+	-	-	-	-	-	-	-	+
12	SP209	+	+	+	+	+	-	-	-	-	-	+
13	SP228	+	+	+	-	-	-	-	-	-	-	+
14	SP237	+	+	+	+	+	-	-	-	-	-	+
15	SP256	+	+	+	-	-	-	-	-	-	-	+
16	SP266	+	+	+	-	-	-	-	-	-	-	+
17	SP271	+	+	+	+	-	-	-	-	-	-	+

Key: '+' = Growth observed, '-'= No growth, '1 ppm'= 0.001 mg/ml.

Table 7: Minimum inhibitory concentration (MIC) of Amoxicillin against *S. pyogenes* isolates at different concentration (ppm).

S.NO	Sample	MIC of Amoxicillin against different isolates at different ppm (mg/ mL)									Control
		0.9 ppm	0.8 ppm	0.7 ppm	0.6 ppm	0.5 ppm	0.4 ppm	0.3 ppm	0.2 ppm	0.1 ppm	
1	SP09	-	-	-	-	-	+	+	+	+	+
2	SP28	-	-	-	-	-	+	+	+	+	+
3	SP49	-	-	-	-	-	+	+	+	+	+
4	SP55	-	-	-	-	-	+	+	+	+	+
5	SP79	-	-	-	-	-	+	+	+	+	+
6	SP92	-	-	-	-	-	+	+	+	+	+
7	SP112	-	-	-	-	-	+	+	+	+	+
8	SP149	-	-	-	-	-	+	+	+	+	+
9	SP157	-	-	-	-	-	+	+	+	+	+
10	SP172	-	-	-	-	-	+	+	+	+	+
11	SP191	-	-	-	-	-	+	+	+	+	+
12	SP209	-	-	-	-	-	+	+	+	+	+
13	SP228	-	-	-	-	-	+	+	+	+	+
14	SP237	-	-	-	-	-	+	+	+	+	+
15	SP256	-	-	-	-	-	+	+	+	+	+
16	SP266	-	-	-	-	-	+	+	+	+	+
17	SP271	-	-	-	-	-	+	+	+	+	+

Key: '+' = Growth observed, '-' = No growth.

Discussion

In this study, about 300 samples were collected from pus, throat and surgical wounds by the aid of sterile syringes and swab sticks and shifted to processing laboratory nearly in sterile environment. By disc diffusion method, Antibiotic susceptibility was checked on MHA plates while in the study of Patil et al. (2013), 1790 different clinical samples of skin pus, throat and surgical wounds were collected with sterile swabs and syringes. By streak plate procedure, samples were inoculated on nutrient agar, blood agar plates. Then, the plates were incubated at 37°C aerobically for twenty-four hrs. Microorganism colonies were confirmed by common place organic chemistry tests. By Kirby-Bauer disc diffusion technique, antimicrobial status tests of the isolates were performed on MHA.

In the present study, from 300 samples, overall 17 positive samples of *S. pyogenes* were isolated and identified. Culture sensitivity was performed to check the resistance pattern of *S. pyogenes* against Penicillin. Penicillin included the following antibiotics: Amoxicillin, Ampicillin, Penicillin G while in the study of Camara *et al.*, (2013) the following antibiotics were used i.e. Ceftriaxone 30 (µg), Amoxicillin (25 µg), Penicillin G (10 µg), Cefpodoxime (10 µg), Cefixime (5 µg), Cefotaxime (30 µg).

From identified 17 positive *S. pyogenes* isolates, the resistant species for Amoxicillin were SP28, SP49, SP55, SP79, SP92, SP112, SP149 according to CLSI, (2016) while the resistant isolates for Ampicillin were SP09, SP28, SP49, SP55, SP79, SP92, and SP112. In the current study the rate of maximum resistance was observed against Penicillin G (76.47) followed by Ampicillin and Amoxicillin (41.17) while less resistance was observed against Piperacillin (29.41) while in the study of Malik *et al.* (2005) many of *S. pyogenes* isolates were liable to Oxacillin and Cephradine, however, several resistant strains were also identified.

In the present study, MIC values were observed at different parts per million (ppm). For Penicillin G, the MIC value for thirteen isolates was 5 ppm (eq. to 0.005 mg/ml) and the value for the remaining four isolates was 4 ppm (eq. to 0.004 mg/ml). Both values fall in resistant category according to CLSI guidelines (2014). In the study of Camara *et al.* (2013), the MIC values were observed between 0.004–0.032 mg/ml. So far, penicillin treatment disasters had listed in patients with tonsilo-pharyngitis, which has been considered as a route for rise of penicillin-resistant strains.

Similarly, for Ampicillin the MIC value for the nine isolates was 4 ppm which falls in intermediate category and 5 ppm for four isolates that ranges in resistant category. However, in the study of Creti *et al.* (2007), the value ranges between 0.015 -0.03 µg / ml. In another study of Rizwan *et al.* (2016) the MIC value was between 0.016 - 0.25 µg / ml respectively.

Decreased Minimum inhibitory concentration values for Amoxicillin were observed at the concentration of 0.4ppm (0.0004 mg/mL) - 0.1ppm (0.0001 mg/mL) which falls in susceptible range for *S. pyogenes* isolates while in the study of Ndiaye *et al.* (2009) the MIC value were between 0.016–0.125 mg/L. On the contrary, in the study of Doern & Brown (2004) the MIC value were observed between 0.016-0.5 respectively.

Conclusions

The present research study was focused on to assess antibiotic susceptibility of Penicillin group of antibiotics against 17 different isolates of *S. pyogenes*. It was concluded from the result that high antibiotic resistance was observed against Ampicillin (82.35%) followed by Penicillin G (76.47%), Amoxicillin (41.17%). Increased minimum inhibitory concentration (MIC) value was observed against Penicillin 7 ppm (0.007 mg/mL) followed by Ampicillin 6 ppm (0.006 mg/mL) and Amoxicillin 4 ppm (0.004 mg/mL).

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