SUSCEPTIBILITY PATTERN OF DIFFERENT ANTIBIOTICS AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT:

Main objective of the study was to evaluate recent sensitivity pattern of *Staphylococcus aureus*. Total of seventy specimens of *Staphylococcus aureus* were collected from different laboratories of Karachi and results were analyzed at Faculty of Pharmacy, Department Of Microbiology, Hamdard University Karachi. The antibiotics tested for sensitivity were Ciprofloxacin (5µg), Levofloxacin (5µg), Imipenem (10µg), Clarithromycin (15µg), and Norfloxacin (10µg). The antibiotic susceptibility test of the bacterial isolates was performed by Kirby-Bauer disk diffusion method [1]. Out of seventy samples 68% samples showed sensitivity against ciprofloxacin, 12.85% showed an intermediate behavior and 18.57% showed resistant towards the ciprofloxacin. For levofloxacin the sensitivity percentage was 72%, Intermediate was 12.85% and resistant was also 12.85%. Norfloxacin had almost similar sensitivity pattern to that of Levofloxacin, Clarithromycin which showed 31.42% resistant 14%, intermediate and 48.57% sensitivity against *S. aureus*. Imipenem which showed 98.57% sensitivity and the resistance was only 1.42%. Which revealed that efficacy of Imipenem, Levofloxacin, Norfloxacin, Ciprofloxacin and Clarithromycin are 98.7%, 74.28%, 72.85%, 68.57% and 48.57% respectively. Imipenem is the most effective antibiotic against *S.aureus* infections.

1. INTRODUCTION:

Repeated use of antibiotics caused to the development of resistant microorganisms [2]. After the evolution of Methicillin resistant Staphylococcus aureus (MRSA) as a nosocomial microorganism in the initial of 1960s, there has been high ratio of uprising of MRSA germs in hospitals recorded from many countries. Lethal septic infection, endocarditis, and osteomyelitis by MRSA have also been recorded. MRSA is frequent nosocomial organism at hospital. Early exposure of MRSA and manufacturing of effective antibiotic strategy in hospitals is of leading cause from epidemiological point of view. [3]

Within each infectious species, sensitivity is different. To check which organism is more sensitive to antibiotic and which antibiotic is more effective for infection in vivo, antibiotic susceptibility test should be carried out. [4] As the use of antibiotics increases in treatment of infections in world, it is necessary to evaluate the antibiotic sensitivity against microorganisms from time to time. Ciprofloxacin showed efficacy in animals and in invitro studies it is effective and safe. [5] Norfloxacin can be used in children for S.aureus infections through oral route. [6] Clarithromycin is a macrolide which shows its effectiveness against keratitis which is caused by S.aureus, it is also useful in many ocular infections. [7] Imipenem is an effective and useful antibacterial medicine for the treatment of S.aureus; it is effective against Gram negative bacteria. Imipenem can be used alone or in combination, it can also be used with gentamicin in combination and shows more effectiveness. [8]

The aim of the study is to evaluate current sensitivity pattern of Staphylococcus aureus, collected from different laboratories and hospitals of Karachi, against various antibiotics namely; Levofloxacin (5µg), Ciprofloxacin (5µg), Imipenem (10µg), Norfloxacin (10µg), Clarithromycin (15µg) to evaluate sensitivity of *S.aureus* by Kirby-Bauer disc diffusion method. [1] Which is a commonly applied method; it is cost effective and gives flexible results. [9] Disc diffusion technique was commonly used by researchers, as this method is easy and results can be obtained by measuring zone of inhibition. [10]

2. MATERIAL AND METHODS:

1.1. Specimen Collection:

This study was performed from November 2014 to June 2015, seventy clinical isolates of *Staphylococcus aureus* were

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collected from different laboratories including Imam Clinic, Essa Lab and Patel Hospital of Karachi. Most of these isolates were obtained from urine samples, some of them were obtained from blood cultures, from urine, wound sample, from otitis media. (Table-1)

1.2. Antibiotic Susceptibility Testing:

Disc diffusion testing was performed according to Kirby-Bauer method, as described in the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [11], using discs containing Levofloxacin (5µg), Ciprofloxacin (5µg), Imipenem (10µg), Norfloxacin (10µg), and Clarithromycin (15µg).

In the laboratory, confirmed colonies for required microorganisms were suspended in 3 mL of tryptone broth by the help of sterilized wire loop in a test tube, to get the uniform growth of microorganisms. After inoculating microorganisms in Tryptone Soya broth, autoclave it for 15 minutes at 121°C. These test tubes were incubated at 37.8°C for half hour. Now this suspension was compared with Macfarland standard then inoculates on the surface of Mueller-Hinton agar (MHA) (OXIOD, UK) which is formulated according to manufacturer, with the help of a sterilized spreader. The inoculums on the plate were streaked with a sterile wire loop. [12] The plates were allowed to dry before applying antibiotic disc. Then, commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours by using Vernier caliper. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were not considered as sensitive in the assessment of data, because it does not give any response in the treatment.

3. RESULTS:

During this study resistance pattern of *Staphylococcus aureus* by applying Ciprofloxacin, Levofloxacin, Norfloxacin, Clarithromycin & Imipenem were calculated. Total seventy clinical isolates of *S.aureus* obtained from urine, throat, wound swab, pus, semen sample, blood, catheter tip from different laboratories of Karachi as described in Table-1. Results exhibited that Imipenem is most sensitive against *S.aureus* infection, it is 98.57% effective. Ciprofloxacin is 68.57% effective, levofloxacin 74.28%, norfloxacin 72.85%, clarithromycin 48.57% shows effectiveness, and detailed figures are present in Table-2. From these results it is obvious that imipenem is most active against *S.aureus* infections and clarithromycin shows somewhat less effect. The graphical representation of resistance of all drugs is available from figure-1 to figure-5 and results are summarized in the table 2.

4. DISCUSSION:

The antibiotic sensitivity pattern in all microorganisms are regularly varying, this research was planned to assess the fresh antibiotic sensitivity pattern of *S.aureus* among general population. To determine the susceptibility of the *Staphylococcus aureus*, Ciprofloxacin, Levofloxacin, Norfloxacin, Clarithromycin & Imipenem antibiotics used. A total of seventy samples were collected from the different laboratories of the Karachi, Sindh Pakistan. These samples were identified from the respective labs. Twenty samples were from pus cell out of which six were from the Imam Clinics, eight from Essa Lab and six from the Patel hospital. Similarly twenty blood samples were collected from these labs which were identified to contain *S. aureus*. Seven from Urine sample, six from the wound swab, six from throat infection, two from the Cather tip and two from Semen. The collection details are in table No. 1.

Antibiotics discs of Ciprofloxacin, Levofloxacin, Norfloxacin, Clarithromycin, and Imipenem were used. Out of seventy samples 68% samples showed sensitivity to the ciprofloxacin, 12.85% showed an intermediate behavior and 18.57% showed resistant against the ciprofloxacin. For levofloxacin the sensitivity percentage was 72%, Intermediate was 12.85% and resistant was 12.85%. Norfloxacin had almost similar sensitivity pattern to that of Levofloxacin, Clarithromycin which showed 31.42% resistant 14% intermediate and 48.57% sensitivity against the *S. aureus*. The last drug was Imipenem which showed 98.57% against *S. aureus* was sensitive and resistance was only 1.42%.

Many researchers described the sensitivity pattern of *S.aureus* against different antibiotics. Onwubiko & Sadiq examined the sensitivity pattern of *S.aureus* against different antibiotics by disk diffusion method, at Nigeria. From different clinics one hundred and fifty clinical isolates collected in 2009 and find *S.aureus* sensitivity ranges to the following antibiotics; Ciprofloxacin (78.9%) and Levofloxacin (100%). Methicillin resistant *S.aureus* organisms are sensitive to Ofloxacin 68.7% and to Levofloxacin 93.7%. [13] In present study levofloxacin is 72% active and ciprofloxacin is 68% active against *S.aureus*.

Taj et al., during the period of January 2009 to December 2009 collected four hundred and fifty isolates of *S.aureus*, in which one hundred and seventy four were *MRSA*. They found resistance against Ciprofloxacin is (63.7%) [14]. In present study resistance against ciprofloxacin is 68%.

Khan et al., reported that; 50 clinical isolates of *Staphylococcus aureus, Proteus, Escherichia coli* and *Klebsiella* obtained from different labs and checked resistance pattern against clarithromycin and erythromycin through using Kirby-Bauer disc diffusion method. In case of erythromycin resistant of *S.aureus* is 27.78% and in clarithromycin resistance is 22.23%. [15] But during this study resistance of *S.aureus* against clarithromycin is 31.42%.

Sarwar et al., described the frequency of *S.aureus* infection at Shaikh Zayed hospital in non operated and operated patients. Fifty samples obtained in which 36% are of non operated patients and remaining are of operated patients. They concluded as the *S.aureus* infection is more common in patients who got surgery rather than those patients which were non operated and found the resistance pattern of *S.aureus* by using different antibiotics included; linezolid, imipenem and tobramycin. They obtained 42% reistance for imipenem [16]. In present study imipenem shows only 1.42% resistance against *S.aureus*.

5. CONCLUSION:

From the above discussion it can be concluded that *S.aureus* showed sensitivity to all the antibiotics which were considered in this study and much resistant to the clarithromycin and more sensitive to Imipenem. In *S.aureus* infection most effective antibiotic is imipenem but its misuse or irrational therapy can lead to develop resistance in *S.aureus*. To diagnose severity of infection and to avoid failure of treatment it is necessary to plan sensitivity pattern against microorganisms from time to time.

Table-1: Distribution of Site of Infection by *S.aureus* Isolates in Hospitals

Site of infection (Number)	Hospital					
	Imam clinic		Essa lab		Patel Hospital	
	No	%	No	%	No	%
PUS (20)	6	20	8	32	6	40
BLOOD(20)	7	23	9	36	4	26
URINE(12)	7	23	2	8	3	20
WOUND SWAB(8)	6	20	1	4	1	6
THROAT (6)	01	3	4	16	1	6
CATHER TIP (2)	1	3	1	4	0	0
SEMEN(2)	2	6	0	0	0	0
Total (70)	**30**	**100%**	**25**	**100%**	**15**	**100%**

Table-2: Antimicrobial Susceptibility Pattern of *S.aureus* on Disc Diffusion

Antimicrobial Susceptibility Pattern Of *S.aureus* On Disc Diffusion			
Name of Antibiotic	**Sensitive No (%)**	**Intermediate No (%)**	**Resistant No (%)**
Ciprofloxacin	48 (68)	9 (12)	13 (18)
Levofloxacin	52 (74)	9 (12)	9 (12)
Norfloxacin	51(72)	10 (14)	9 (12)
Clarithromycin	34 (48)	14 (20)	22 (31)
Imipenem	69 (98)	0	1(1)

Figure.1

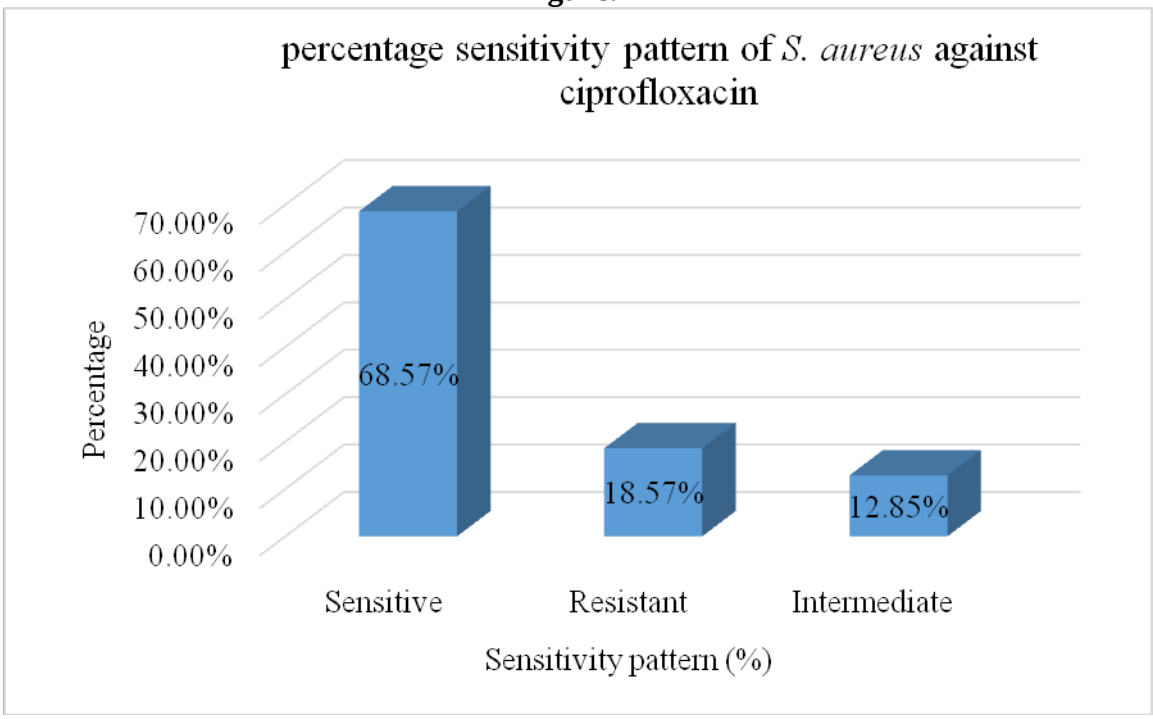


Figure. 2

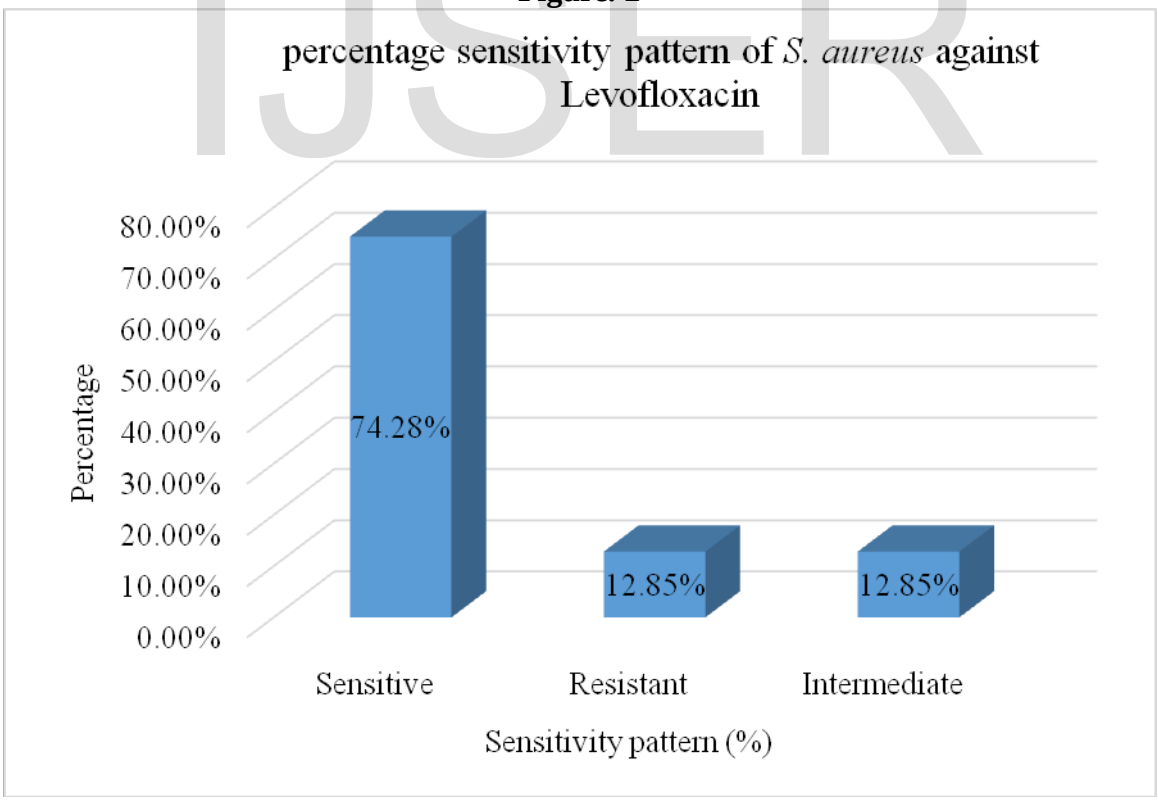


Figure. 3

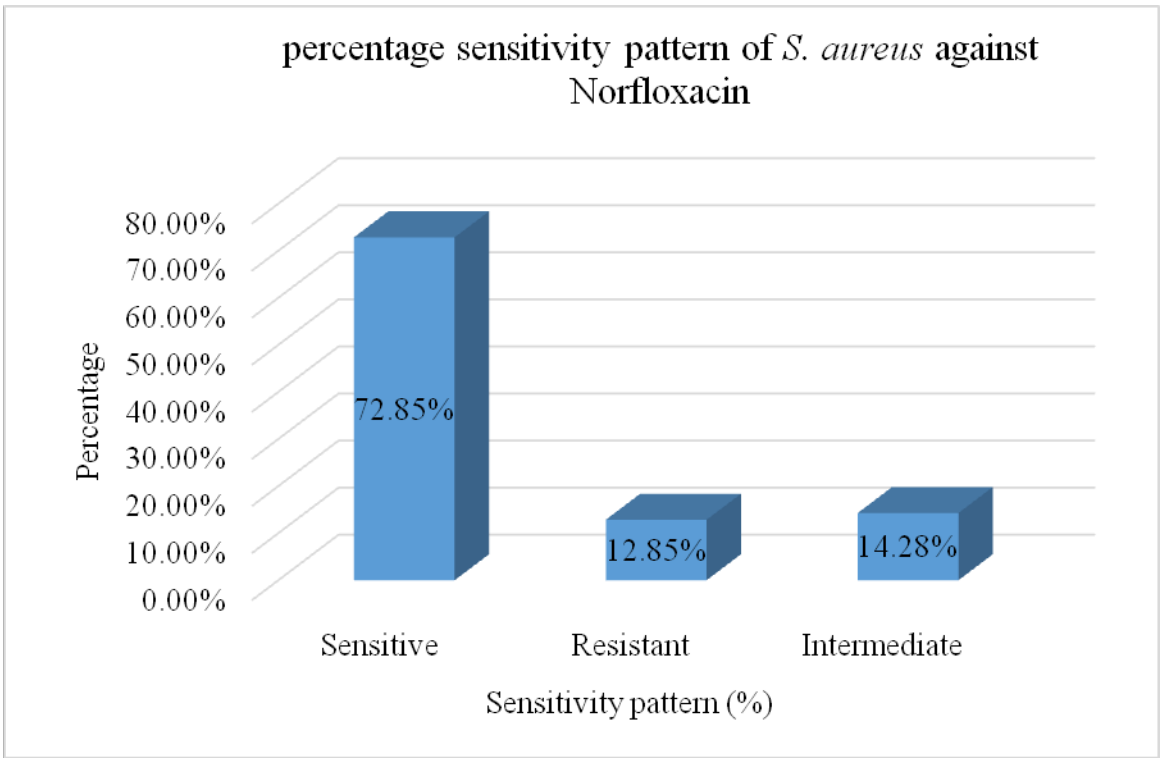


Figure. 4

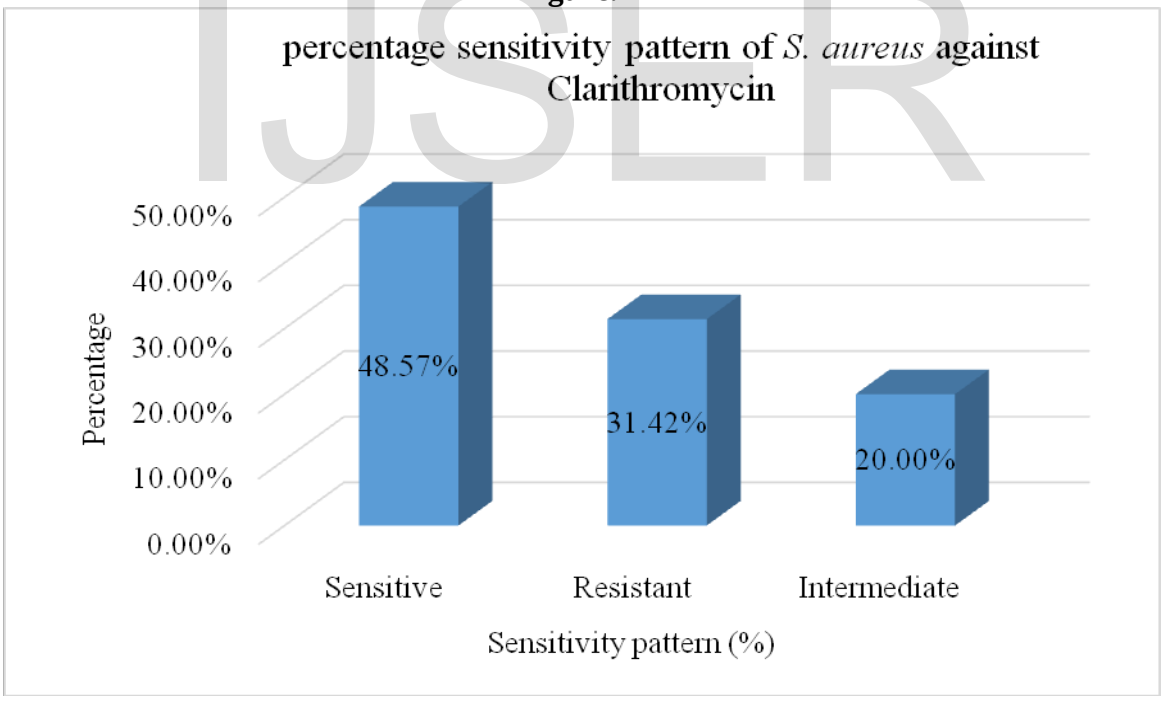
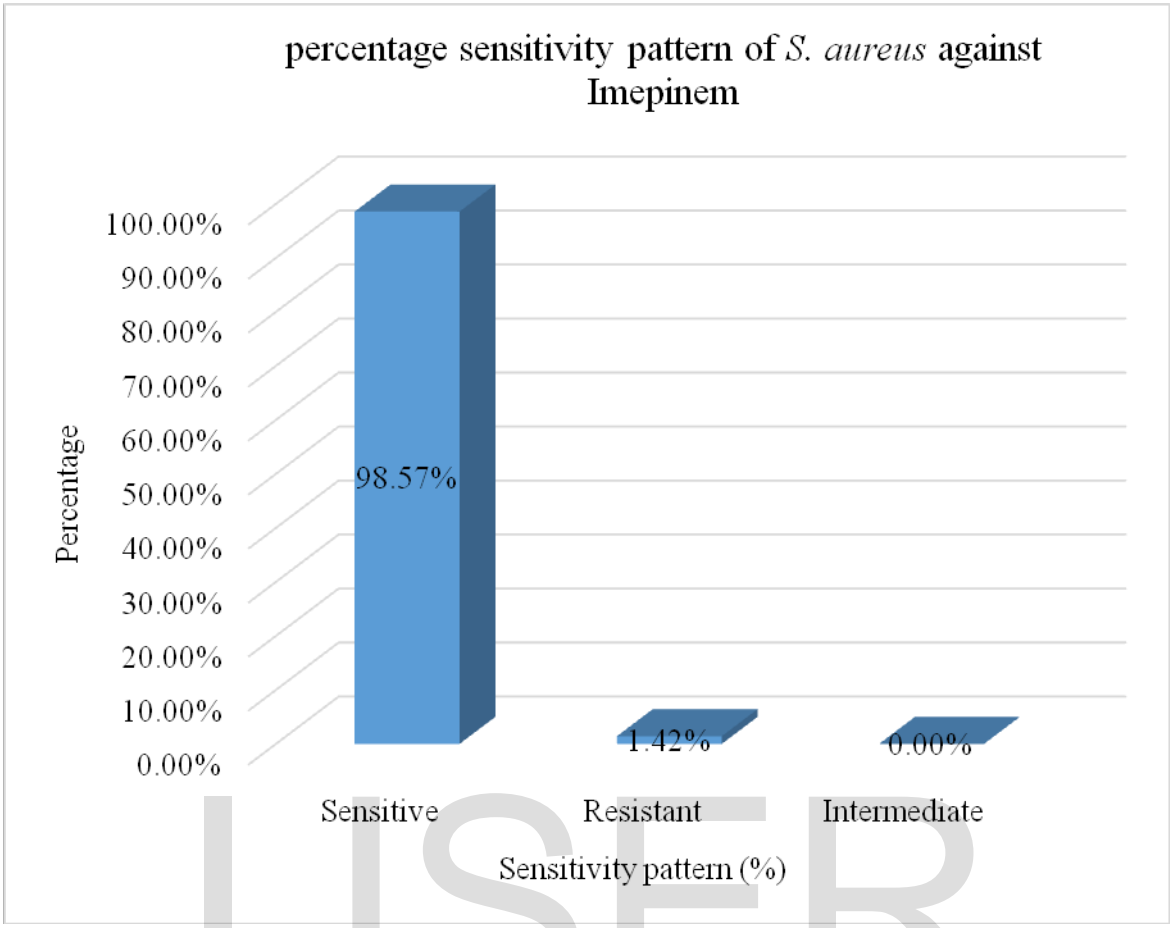


Figure. 5


percentage sensitivity pattern of S. aureus against Imepinem
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Percentage
98.57%
1.42%
0.00%
Sensitive
Resistant
Intermediate
Sensitivity pattern (%)

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