Original Article

Study of Antibiogram and Resistance Mechanism of Staph. Aureus in Clinical Isolates from Stand alone Diagnostic Centre in Central Madhya Pradesh

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Reprint Request
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Abstract

Background: In recent years,Staph.aureus,both coagulase positive and negative,have shown resistance to commonly used antibiotics used to treat infections.Over the last two decades,methicillin resistant strains (MRSA) have also been on a rise specially in patients admitted to ICUs and immunocompromised patients.The drug resistance mechanism of MRSA has been studied extensively in the past decade.Our study aims to study the sensitivity pattern of Staph. aureus in different clinical specimens and to study thedifferent resistance mechanisms**.** *Objective:* The present retrospective study highlights the susceptibility pattern and resistance mechanism ofStaph.aureus in clinical specimens obtained in our Microbiology department from June 2015 to June 2016. *Materials and Methods:* This was a retrospective study of Staph.aureus isolates from different clinical specimens including urine, blood, pus, vaginal swab, semen, aural swab, BAL fluid, conjunctival swab etc obtained from out patients at our Diagnostic Clinic Microbiology department from June 2015 to June 2016 and reported in VItek II (Biomerieux) according to CLSI guidelines. A total of 278 samples were reviewed. *Results:* Out of278 Staph.aureus isolates grown in the lab from different clinical specimens the sensitivity pattern showed highest sensitivity for Tigecycline(91.7%),followed bypenicillin (91.3%), ,Gentamycin (80.9%),tetracycline (66%),levofloxacin(65.4%),Rifampicin(63.6%), Linezol id(63.3%), Daptomycin(50.3%), Vancomycin (46,7%), Teicoplanin (42%). 117 cases showed resistance to aminoglycosides by KAN (APH(3")-III) mechanism,Cefoxitin screen was positive in 166 patients,APH(3")-III in 117 patients,MecA gene in 113 cases,ANT(4′)(4"))In 106 patients,acquired penicillinase in 77 ,SGA-SGB in 49 cases,efflux mechanism in 30 cases and inducible clindamycin resistance in 24 cases. *Conclusion:* According to our study Tigecycline, followed by Penicillin, Gentamycin ,Tetracycline, Levofloxacin and Linezolid are the antibiotics of choice for treating Staph aureus infections in the present scenario. With the introduction of automated equipments like Vitek II our understanding of the resistance mechanism is increasing.A larger study population would be required for a better understanding of resistance mechanisms.

Keywords: Staph Aureus; Vitec II; Resistance Mechanism; MRSA; Meca Gene.

Introduction

The genus Staphylococcus are ubiquitous gram positive, one micron in diameter, non –sporing cocci occurring in grape like clusters, singly or in pairs. They are facultative anaerobes, grow well on blood agar as golden yellow colonies [1]. Staphylococcus aureus causes boils, bronchopneumonia, carbuncles, diabetic foot, infection, furuncles, osteomyelitis, post operative infections, septicaemia and a host of other infections [2,3,4].

In recent years, Staph.aureus,both coagulase positive and negative, have shown resistance to commonly used antibiotics used to treat infections,it causes.Over the last two decades, methicillin resistant strains (MRSA) have also been on a rise specially in patients admitted to ICUs and immunocompromised patients [5].The drug resistance mechanism of MRSA has been studied extensively in the past decade.There are several other resistance mechanisms playing a role in resistance of antibiotics to Staph aureus. Our study aims to study the sensitivity pattern of Staph. aureus in different clinical specimens and to study the different resistance mechanisms.

Material and Methods

This was a retrospective study of Staph.aureus isolates in different clinical specimens including pus, sputum, urine, blood, aural swab, BAL fluid, conjunctival swab, CSF, Nasal swab, parotid fistulas, pleural fluid, Semen, synovial fluid,throat swab and vaginal swab obtained from out patients at our Clinic Microbiology department from June 2015 to June 2016 and reported in fully automated Vitek II (Biomerieux) according to CLSI guidelines. A total of 278 samples were reviewed. All samples whether urine, pus etc. were considered in the study. The patients were divided into four groups ie. Newborn(NB) to 20 years, 21-40 years ,41-60 years, 61 to 80 years and more than 80 years in both the sexes. The following points were taken into consideration for analysis:

- Age and sex of patients
- Staph.aureus i isolates
- Drug sensitivity pattern
- Resistance mechanism

Samples were processed and identified as per routine laboratory protocol. Identification and antibiotic sensitivity testing was done byVitecII (Biomerieux) according to clinical laboratory standard institute guidelines (CLSI guidelines)

Isolation and Identification

Urine samples were collected in universal container approx. 50 ml in amount and were inoculated using an inoculation loop of 10 ul volume calibration on MacConkey agar plates. Other specimens such as CSF, Sputum, and different body fluids collected in sufficient amount were inoculated on Blood and MacConkey agar plates using an inoculation loop. Blood samples collected in broth in a ratio of 1:5 (blood: broth) were incubated in BactT/Alert (Biomeriux) and then subcultured on blood and MacConkey agar plates on the basis of colony morphology , gram staining, motility P628 panel was selected for identification and sensitivity of the micro organism. Following criteria was used for identification of Staph. aureus

1. Colony morphology:-.1 micron diameter,golden yellow colonies

2. Grams Staining :- Gram positive cocci, size, uniformly stained , non sporing , non capsulated

3. Biochemical reaction:- performed on automated VitecII(Biomerieux)

4. Antimicrobial sensitivity tests:- performed on automated Vitec II(Biomerieux)

Results

The present study was conducted in total of 278 Staph aureus isolates from June 2015 to June 2016 through automated identification and sensitivity reporting by VitecII(Biomerieux).

The antimicrobial resistance pattern assessment revealed that out of 278 Staph. aureus isolates there were 60.4% males and 39.5% females.Male to female ratio was 1.52:1.Maximum patients were below 20 years of age(33%),followed by 21-40 years(30.5%), 19.5% in 21-40 years age group. 2.55% patients were above 80 years of age.Demographic data of patients is shown in Table 1.

Majority of Staph aureus isolates from pus(55%), followed by sputum(16.5%),throat swab (14.0%), blood(3.59%),semen(3.23%)urine(1.79%), conjunctival swab and CSF (1.07%), synovial fluid, pleural fluid and BAL fluid (0.7%) and lowest in parotid fistula, nasal and aural swab (0.35%).The data of Staph aureus isolated in different clinical specimens is shown in Table 2.

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The sensitivity pattern showed highest sensitivity for Tigecycline (91.7%), followed by penicillin (91.3%), Gentamycin (80.9%), tetracycline (66%), levofloxacin (65.4%), Rifampicin (63.6%), Linezolid (63.3%), Daptomycin (50.3%), Vancomycin (46,7%), Teicoplanin (42%). Lowest sensitivity was found in ampicillin (2.15%)and amoxicillin (2.5%).Table 3 shows the MIC value and sensitivity pattern of antibiotics Ampicillin showed highest resistance (97.8%), followed by amoxicillin (97.5%), ofloxacin (89.57%), erythromycin(73.1%), ciprofloxacin (76.7%), ampicillin/sulbactum(71.6%), ceftriaxone and cefotaxime(71.3%)each, cefazoline (69.8%), Imipenem (70.9%).

Table 4 shows resistance pattern and MIC value of antibiotics.

117 cases showed resistance to aminoglycosides by KAN (APH(3')-III) mechanism. Out of these 117 cases, 61 cases were from pus, 16 from sputum,14 from throat swab, 7 from blood, 4 in urine, 3 in conjunctival swab and CSF, 2 in BAL fluid,one each in aural, nasal and vaginal swab, synovial and pleural fluid, parotid fistula and semen.

106 cases shows resistance to aminoglycosides by KAN TOB (ANT(4')(4")) mechanism.out of which 61 cases were from pus, 16 from sputum 14 from throat swab and seven from blood.

Resistance by acquired penicillinase mechanism to B-lactams was observed in 77 cases, with 53 cases

in pus, 10 in throat swab, 6 in sputum, 4 in blood.

In the family of Macrolides/lincosamides/streptogramins 30 cases showed resistance to antibiotics by efflux mechanism,out of which 18 were from pus isolates, 3 each from sputum and throat swab and 2 from blood.

Resistance to Streptogramins by SGA-SGB was observed in 49 cases, 30 from pus, 6 from sputum,5 each from blood and throat swab.

Inducible Clindamycin resistance was observed in 224 cases,18 in pus, 3 in throat swaband 2 in sputum.

Cefoxitin screen was found to be positive in 166 cases, 77 in pus, 36 in sputum, 24 in throat swab 9 in semen.

PBP (MecA) gene was observed to cause resistance in B lactam antibiotics in 113 patients,out of which 53 were from pus,16 from sputum13 from blood 11 from throat swab .

Cefoxitin screen was positive in 166 patients, APH(3")-III in 117 patients, MecA gene in 113 cases,ANT(4!)(4"))In 1q06 patients, acquired penicillinase in 77, SGA-SGB in 49 cases, efflux mechanism in 30 cases and inducible clindamycin resistance in 24 cases.

Table 5 shows the data of resistance mechanism in different clinical specimens.

Table 1: Demographic data of stapylococcus aureus

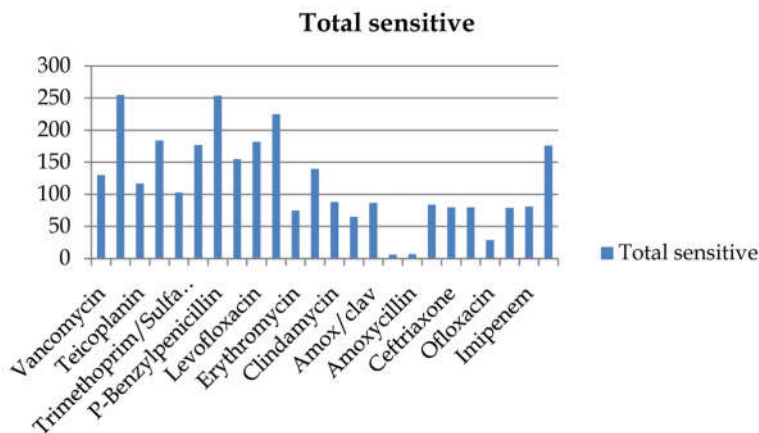| Age (Years)	Male	Female	Total
<20	55	37	92
21-40	51	34	85
41-60	32	22	54
61-80	28	12	40
>80	2	5	7
Total	168	110	278
Percentage	60.40%	39.50%	
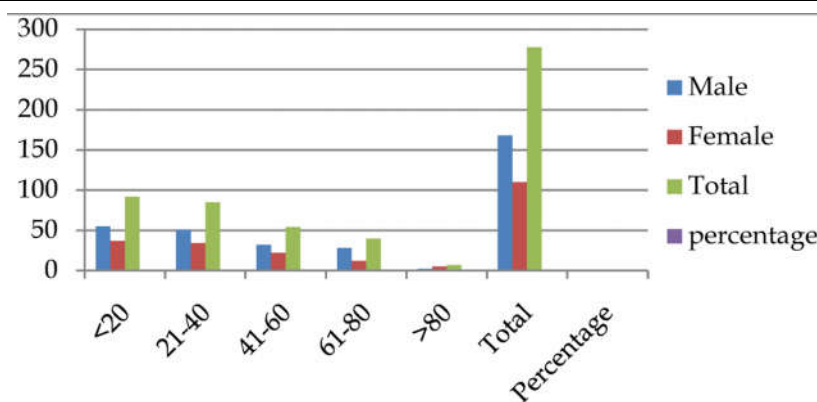
Graph 1: Showing demographic data
Isolation of Staph.aureus in different clinical specimens

Table 2: Showing Staph Aureus isolates from different clinical specimens

Specimen	Male	Female	Total	Percentage
Pus	93	60	153	55%
Sputum	27	19	46	16.50%
Urine	4	1	5	1.79%
Blood	4	6	10	3.59%
Aural Swab	1	0	1	0.35%
BAL	1	1	2	0.70%
Conjuctival Swab	1	2	3	1.07%
CSF	2	1	3	1.07%
Nasal Swab	1	0	1	0.35%
Parotid Fistula	1	0	1	0.35%
Pleural Fluid	1	1	2	0.70%
Semen	9	0	9	3.23%
Synovial Fluid	1	1	2	0.70%
Throat Swab	23	16	39	14.00%
Vaginal Swab	0	1	1	0.35%
Total	169	109	278	
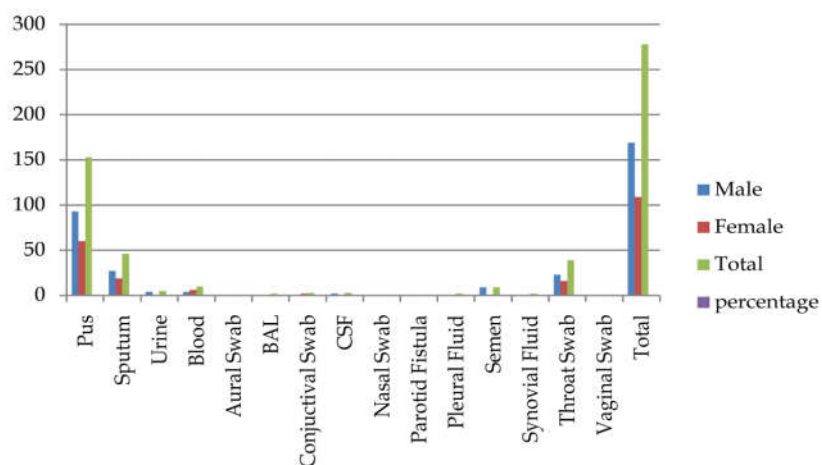
Graph 2: Showing Staph Aureus isolates from different clinical specimens

Table 3: Sensitivity pattern of staphylococcus aureus in various clinical specimens

Antibiotic	Total sensitive	percentage	MIC Value
Vancomycin	130	46.70%	<=0.5
Tigecycline	255	91.70%	<=0.12
Teicoplanin	117	42%	<=0.5
Tetracycline	184	66%	<=1
Trimethoprim/Sulfamethoxazole	103	37%	<=10
Rifampicin	177	63.60%	<=0.03
P-Benzylpenicillin	254	91.30%	0.12,
Oxacillin	155	55.70%	<=0.25
Levofloxacin	182	65.40%	0.25
Gentamicin	225	80.90%	<=0.5
Erythromycin	75	26.90%	<=0.25
Daptomycin	140	50.30%	0.25,
Clindamycin	88	31.60%	0.25
Ciprofloxacin	65	23.30%	<=0.5
Amox/clav	87	31.20%	<= 0.5
Ampicillin	6	2.15%	<= 0.5
Amoxycillin	7	2.50%	<=0.25
Ceftazoline	84	30.20%	<=0.25
Ceftriaxone	80	28.70%	<=0.5
Cefotaxime	80	28.70%	<= 0.5
Ofloxacin	29	10.43%	<=0.25
Ampicillin+Sulbactum	79	28.40%	<=0.25
Imipenem	81	29.10%	<=0.25
Linezolid	176	63.30%	<=0.5

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Graph 3: Showing sensitivity pattern

Table 4: Resistance pattern of Staph.aureus in different clinical specimen

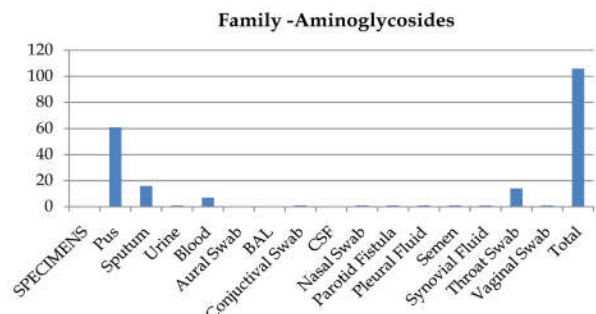| Antibiotic	Total Resistant	MIC Value	Percentage
Vancomycin	148	>=32	53.30%
Tigecycline	23	1	8.30%
Teicoplanin	161	>=32	58%
Tetracycline	94	>=16	33.90%
Trimethoprim/Sulfametho xazole	175	>=320	63%
Rifampicin	101	>=4	36.40%
P-Benzylpenicillin	24	>=0.5	8.70%
Oxacillin	123	>=4	44.30%
Levofloxacin	96	>=8	34.60%
Gentamicin	53	>=16	19.10%
Erythromycin	203	>=8	73.10%
Daptomycin	138	>=8	49.70%
Clindamycin	190	>=4	68.40%
Ciprofloxacin	213	>=8	76.70%
Amox/clav	191	>=8	68.80%
Ampicillin	272	>=8	97.80%
Amoxycillin	271	>=8	97.50%
Cefazoline	194	>=4	69.80%
Ceftriaxone	198	>=8	71.30%
Cefotaxime	198	>=8	71.30%
Ofloxacin	249	>=16	89.57%
Ampicillin+Sulbactum	199	>=8	71.60%
Imipenem	197	>=8	70.90%
Linezolid	102	>=8	36.70%
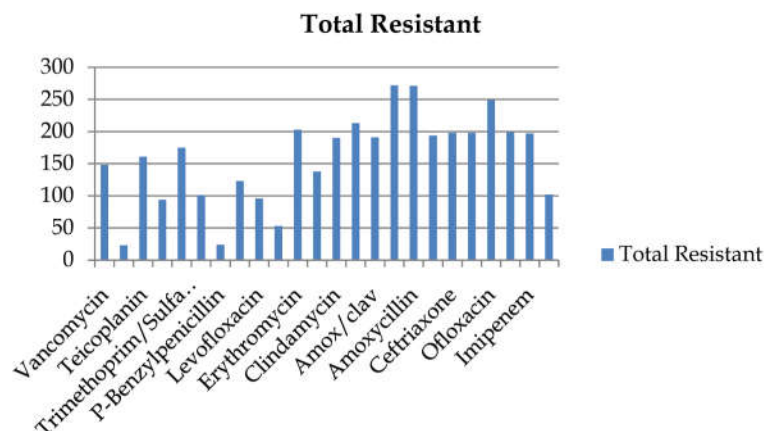
Graph 4: Showing resistance pattern of Staph aureus

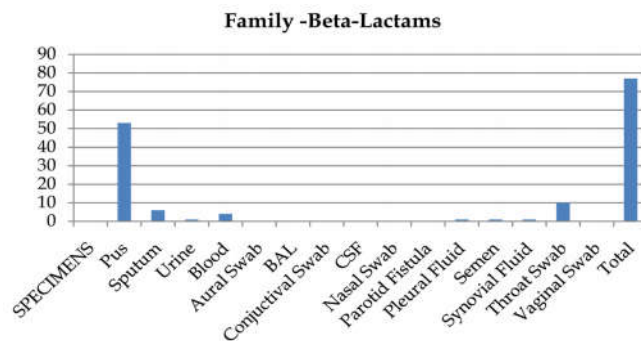**Table 5:** Showing resistance mechanism of Staph aureus in different clinical specimens

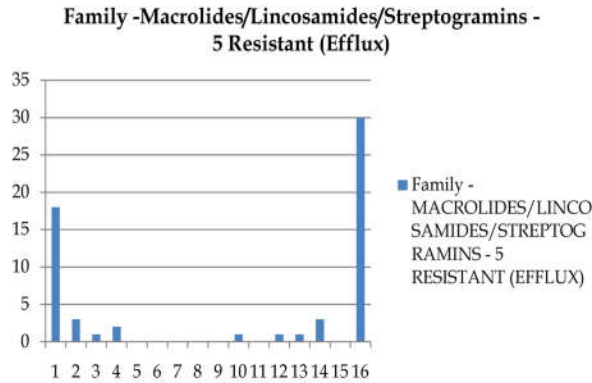| SPECIMENS	Family - AMINOGLYCOSIDES – 18 RESISTANT KAN (APH(3')-III)	Family - AMINOGLYCOSIDES – 19 RESISTANT KAN TOB (ANT(4')(4")	Family - BETA-LACTAMS – 6 ACQUIRED PENICILLINASE	Family - MACROLIDES/LINCOSAMIDES/STREPTOGRAMINS RESISTANT (EFFLUX)	Family - MACROLIDES/LINCOSAMIDES/STREPTOGRAMINS – 8 RESISTANT TO STREPTOGRAMINS (SGA-SGB)	Family - TRIMETHOPRIM/SULFONAMIDES – 1 RESISTANT	Family - TRIMETHOPRIM/SULFONAMIDES – 2 TRIMETHOPRIM RESISTANT	ICR-Inducible Clindamycin Resistance POSITIVE	OXSF-Cefoxitin Screen POSITIVE	Family - BETA-LACTAMS – 21 Modification of PBP (mecA)
Pus	61	61	53	18	30	69	25	18	77	53
Sputum	16	16	6	3	6	9	9	2	36	16
Urine	4	1	1	1	0	3	1	0	4	3
Blood	7	7	4	2	5	3	6	0	3	13
Aural Swab	1	0	0	0	0	0	0	0	1	2
BAL	2	0	0	0	0	0	0	0	2	0
Conjuctival Swab	3	1	0	0	1	0	0	0	3	2
CSF	3	0	0	0	0	0	0	0	3	1
Nasal Swab	1	1	0	0	1	0	1	0	1	1
Parotid Fistula	1	1	0	1	0	0	1	0	1	1
Pleural Fluid	1	1	1	0	1	0	0	0	0	1
Semen	1	1	1	1	0	3	2	1	9	7
Synovial	1	1	1	1	0	0	0	0	1	0
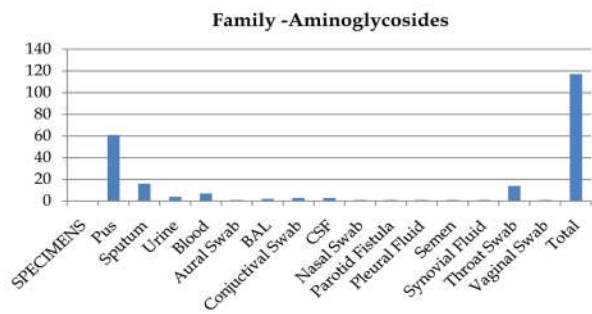
Graph 5: Resistance mechanism RESISTANT KAN (APH(3')-III)

Graph 6: Resistant Kan Tob (ANT(4')(4"))

Graph 7: Acquired penicillinase

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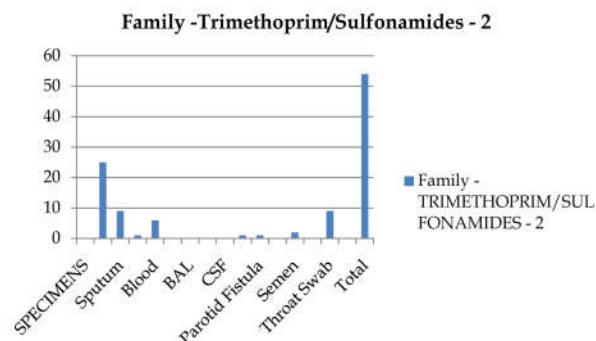
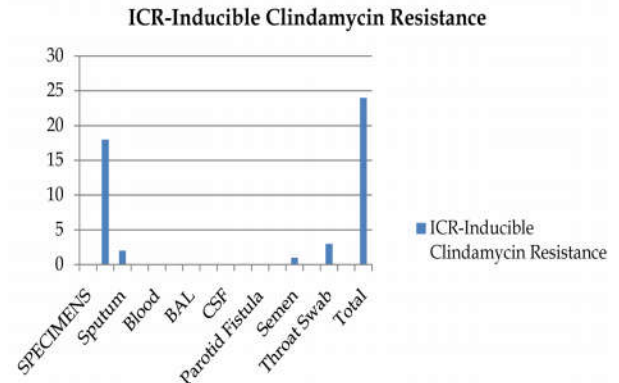103

Graph 8: Resistant (efflux
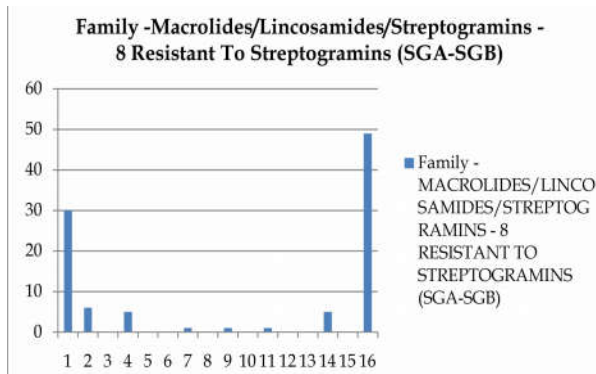
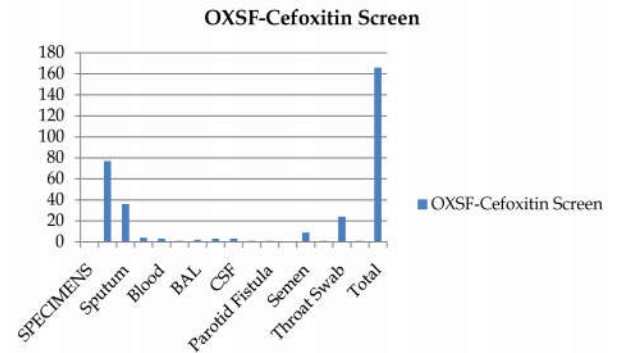**Graph 9:** Resistant to streptogramins (SGA-SGB)
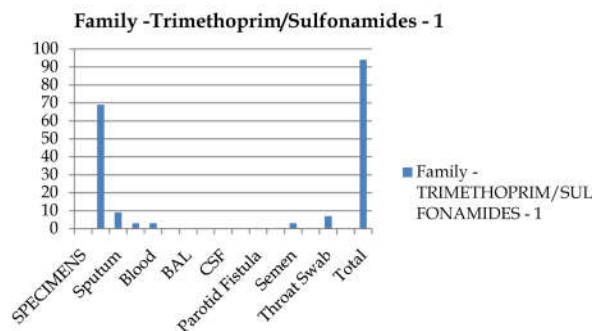
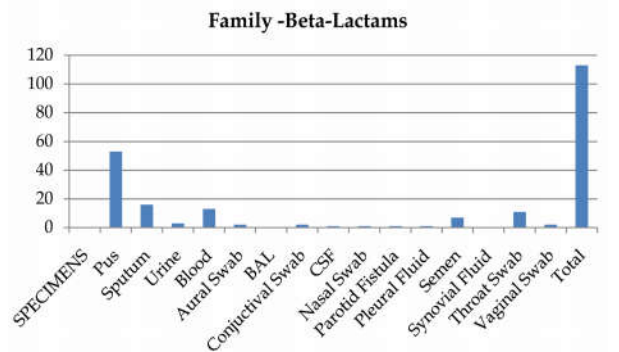**Graph 10: Family-Trimethoprim/Sulfonamodes-1**

Graph 11: Family-Trimethoprim/Sulfonamodes-2

Graph 12: ICR-Inducible Clindamycin Resistance

Graph 13: Cefoxitin screen positive

Graph 14: Modification of PBP (mecA)

Discussion

Drug resistance in Staph aureus is a major global health problem.It increases the morbidity and mortality among the patients and reduces the chances of using sensitive drugs for future generations.It also adds to the economic burden for healthcare systems.

The rate and magnitude of drug resistance in Staph aureus is mediated by a complex interplay of different epidemiological factors and mechanisms. Overuse and misuse of antibiotics is one of the reasons of resistance.

There are several mecjhanisms by which antibiotics act on the microbes e.g. drugs such as Aztreonam, cefalosporins, penicillins, Vancomycin, Imipenem and Methicillin act by inhibiting the cell wall synthesis of bacteria.Some antibiotics act by inhibiting enzymes involved in DNA synthesis like Quinolones. Drugs like sulphonamides inhibit tetrahydrofolic acid needed for DNA synthesis.Aminoglycosides,Cloramphenicol act by inhibiting protein synthesis [6].

Drug resistance among bacteria develops as a result of mutations in the microorganism's genetic structure or by acquiring extra pieces of genetic material from other bacteria. There are several drug resistance mechanisms like(a) Decreased drug uptake modification of plasma membrane causing reduced permeability, (b)increased drug export caused by increased activity of efflux pumps, (c)inactivation or modification mutations in ribosomal proteins, penicillin binding proteins(PBP), (d) Introduction of new drug-insertion of methicillin resistance gene(MecA), (e)increased production of Beta lactamase gene [7]. Cross resistance develops between members of a class of antibiotics because they are chemically related and have the same target of action in bacterial cells .The The drug efflux mechanism confers resistance to betalactams,aminoglycosides, tetracyclines, macrolides, streptogramins etc.The intracellular antibiotic concentration is reduced by the efflux mechanism thereby delaying the death of bacterium.Absence of or alteration in aminoglycoside transport system, inadequate membrane potential, modification in lipopoly saccheride (LPS) phenotype can result in a cross resistance to all aminoglycosides. The enzymes causing inactivation of aminoglycosides are classified according to the type of modification AAC (acetyltransferases), ANT (nucleotidyl transfereases) or adenyltransferases, APH (phosphotransferases) (Shaw et.al 1993) [8].

Penicillin was introduced in early 1940s and soon developed resistance due to the ability of Staph aureus to produce Beta lactamase enzyme i.e. penicillinase.Penicillin converts the beta lactam nucleus into harmless peniciloic acid. MRSA worsened this situation.Methicillin was introduced in 1961 as it was penicillinase stable beta lactam antibiotic., but since then, MRSA strains have become endemic [9]. MRSA contains MecA gene which is responsible for the production of penicillin binding protein(PBP 2a) [10].

Staph aureus also develops resistance due to NorA multidrug resistance efflux pump resulting in low level quinolone resistance [11].

In the study conducted by Alain C et al. in 2014, 40.6% cases were identified as MRSA and 39.4% were inducible Clindamycin resistance. The found 100% sensitivity for Linezolids followed by tetracycline(95%),while Penicillin G had 0% sensitivity [12]. Our study does not correlate with this study. Our study showed 91.7% sensitivity for Tetracycline, 91.3% for Penicillin, Linezolid(63.3%) and inducible clindamycin resistance in only 24 cases.

In the study of Uwaezuoke et al. ,high sensitivity was found to Gentamycin(91.7%), Cloxacillin (85.4%), Erythromycin(66.7%), Streptomycin (66.7%) [13].Our study does not correlate with this study as well. Emmanuel et. al. found highest sensitivity to Levofloxacin(100%), followed by Ciprofloxacin (78.9%) and least to Penicillin (7.1%) [14]. Najim Abdulla et al. found Amikacin, Gentamycin And Doxycycline to be highly susceptible [15]. Lowest rates were seen with Amoxycillin, Amoxyclav,Erythromycin, Cotrim-oxazole and Cefuroxime. Our study partly correlates with this study. Mazhar Salim et al. found highest sensitivity to cloramphenicol, Linezolid, Nitrofurantoin, Rifampicin and Teicoplanin but high resistance to Erythromycin and Penicillin. All isolates were sensitive to Vancomycin [16].

Conclusion

A continuous surveillance of antibiotic sensitivity pattern and resistance mechanism is needed for selecting appropriate antibiotic therapy for Staph aureus in different clinical specimens. According to our study Tigecycline, followed by Penicillin, Gentamycin, Tetracycline, Levofloxacin and Linezolid are the antibiotics of choice for treating Staph aureus infections in the present scenario.Ampicillin and Amoxycillin have caesed to be the first line drugs for treating Staphylococcal infections. Multidrug resistance IN Staph aureus is an alarming sign. Newer approaches to therapy and prevention are required to combat this problem. With ithe introduction of automated equipments like Vitek II our understanding of the resistance mechanism is increasing. A larger study population would be required for a better understanding of resistance mechanisms.

Conflict of Interest

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