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Antibiotic Susceptibility Pattern of Clinical Isolates of Methicillin Resistant Staphylococcus Aureus in Peshawar, Pakistan

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Abstract:

The Staphylococcus aureus are facultative anaerobic gram-positive cocci that arrange in grape like bunch of irregular clusters. On the base of enzyme coagulase, the organismhaspotential to clot blood plasma; staphylococci are separated into coagulase positive staphylococci and coagulase negative staphylococci. Coagulase-positive staphylococci include pathogenic organism named *S. aureus.* MRSA is recognized as a major cause of nosocomial infections ranging from minor skin infection to life threatening disease. The emergence of MRSA strains is

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an alarming situation for both hospitalized and non-hospitalized patients.The current study was aimedto find the prevalence and antibiotic susceptibility pattern of MSSA and MRSA from clinical isolates. A total of 100 clinical isolates of *Staphylococcus aureus* was collected from indoor patients of Khyber Teaching Hospital Peshawar. The isolation and identification of these clinical isolates were done using standard microbiological methods as per CLSI guidelines. The Antibiotic susceptibility pattern was done by using disk diffusion method. Out of 100 clinical isolates, 18(18%) were MRSA and 82(82%) were MSSA. Prevalence of MRSA was same in male and female. MSSA showed higher susceptibility to doxycycline (98.0%) while AMC (29.0%) showed lowest susceptibility against *S. aureus*. MRSA was higher sensitive to Vancomycin and Amikacin (94.4%) but resistant to cefoxitin (100%). Thecurrent study concludes that 18% isolates were MRSA in indoor patients of MTI KTH while the remaining isolates were MSSA.

Keywords: MRSA, Bacteria, MSSA, Antibiotics, Peshawar, coagulase, Staphylococcus aureus

Introduction:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the extremely frequent cause of nosocomial infections around the world. The World Health Organization (WHO) must be classified MRSA in accordance with a global high priority pathogen list to help promote research and development the pipeline for the finding of novel antimicrobials [1]. Staphylococcus aureus are facultative anaerobic gram-positive cocci, usually arranged in grape like bunch of irregular clusters. The S. aureus are classified according to activity of enzyme coagulase the organism has ability to clot blood plasma, staphylococci are separated into coagulase positive staphylococci and coagulase negative staphylococci [2]. Coagulase-positive staphylococci include pathogenic organism named *S. aureus*. In the early 1960s, beta-lactamase resistant semi-synthetic penicillin became available for clinical use; it was effective for a while but ended with the emergence of Methicillin (oxacillin) Resistant *Staphylococcus aureus* (MRSA) [3]. Worldwide, Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major a etiology of nosocomial infections. The emergence of MRSA is an alarming situation in the community for both hospitalized and non-hospitalized patients. Earlier studies have found that such infections have distinctive strain, virulence, and epidemiologic properties [4,5]. MRSA causes some serious infections which is a

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great concern for the clinicians, as the mortality and morbidity of disease is quite high [6]. The irrational use of antibiotic is one of the major causes of MRSA infection and prolonged hospital stay. [7]. Reservoirs are the Infected and colonized patients and primarily it transmits through contaminated hands [8]. there number of MRSA strains are resistant to antimicrobial drugs, but the glycopeptide antibiotic is still working against the resistant strains of S. aureus including vancomycin [9] Staphylococcus aureus and MRSA are normally found on the skin and mucous membranes of healthy individuals; thus, humans are the major reservoir for these organisms [10,11]. According to research, it is estimated that approximately half of all adults are colonized, and up to 15% of the population persistently carry *S. aureus* in the anterior nares. Some individuals are more vulnerable to higher rates of S. aureus colonization (up to 80%), such as health care workers, persons who use needles on a regular basis (i.e., diabetics and intravenous (IV) drug users), hospitalized patients, and immune compromised individuals. *Staphylococcus aureus* can be transferred from one person to another either by direct contact, by infected droplets from sneezing or coughing, or by fomites [12,13,14]. Staphylococcus aureus found in the normal flora of intact skin. *S. aureus*persist as a hospital acquired and community-acquired pathogen and causes public health concern. Mucous membranes have recently got attention as a potential pathogen, specifically for nosocomial infections [15,16]. *S. aureus* is important pathogenic bacterium responsible for minor skin infections such as folliculitis, furuncles, andabscesses to life-threatening diseases such as meningitis, pneumonia, endocarditis, toxic shock syndrome (TSS), and septicemia [17,18]. The aim of current to find the prevalence of MRSA isolated from clinical patients Isolation and identification of MRSA from clinical isolates of infected patients and observe the antibiogram of *S. aureus* against selected antibiotics in KTH hospital Peshawar.

Methodology:

Study Site:

This study was conducted in department of pathology at KTH Peshawar. from December 2019 to March 2020 in microbiology section.

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Sample collection:

A total 650 clinical samples were collected from indoor patients. Different types of specimens were collected including pus, fluids, sputum, and blood collected in sterilized bottles and swabs are processed in microbiology laboratory in Khyber teaching hospital (KTH). These collected samples were labeled properly.

Isolation and identification of clinical specimens:

All the samples of *S. aureus* were cultured on MacConkey and sheep blood agar and incubated for 24hrs at 37C for the bacterial growth. These bacterial isolates were then subjected by performing standard microbiological techniques such as Gram staining and biochemical tests. The isolates were further processed to culture sensitivity by disc diffusion method using different selected antibiotics on nutrient agar.

Media preparation

Media sterilization was done by using autoclave at temperature 121cand maintained for 15 to 20 mints. The media used for culturing are blood agar and MacConkey agar, Nutrient agar, Nutrient broth media. MacConkey agar is differential and selective medium used for the isolation and differentiation of gram-negative rods. It differentiates lactose and non-lactose fermenting bacteria the procedure of composition as shown in Table.No.1.

Table.No.1: Composition of MacConkey Agar:

Peptone	20.0
Bile salt	31.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
PH	7.1+-0.2at 25C

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Gram staining:

Gram staining had been done for microscopic analysis which is differential method for differentiation of gram negative and gram-positivebacteria. Water drop was put on slide and with the help of loop colony was streaked onto the slide in circular motion to make a thin layer. The smear was heat fixed. Crystal violet dye was applied on the heat fixed smear and remained for 60 seconds and was then washed under running tap water.Then the slide was flooded with iodine solution and remain for 60 seconds, and the slide was then washed with tap water.Decolorized with alcohol for 15 seconds and again washed off.Safranin (counter stain) was added for 30 seconds giving red\pink color to the background. Gram positive and negativebacteria as shown in Figure.No.1,

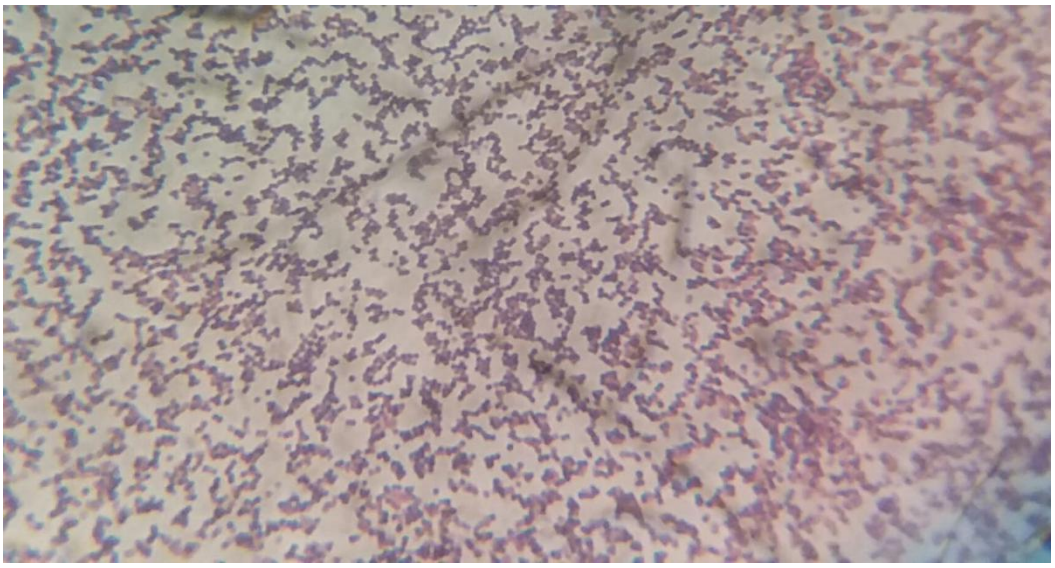

Figure.N0.1: Gram staining of positive and Negative Bacteria

Biochemical tests:

For further identification and chemical analysis of positive isolates, following biochemical tests were performed: Catalasetest, Coagulase test, MSA (Mannitol salt agar).

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Catalase test:

Catalase is an enzyme that break down hydrogen peroxide into water and oxygen. Thecatalase test is used to find out the catalyze enzyme presence.On a clean slide transfer a small amount of bacterial colony, dry glass slide using inoculating loop. Positive result showed bubbles production.while Negative results showed no production of bubbles.

Coagulase test:

Coagulase test is used to differentiate staphylococcus aureus (positive) from coagulase negative staphylococcus. Coagulase is an enzyme and cause plasma to clot by converting fibroin (soluble) to fibrin (insoluble). A drop of saline on to the slide.With the wire loop transfer small amount of bacterial colony on a slide in each drop to make two thick suspensions.Add drop of human plasma into the staphylococcus aureus on a slide for clumping of the organism within 10 seconds.

Mannitol salt agar (MSA):

Mannitol salt agar (MSA) is used as a selective and differential medium for isolation of staphylococcus aureus from clinical and non-clinical specimens.Mannitol salt agar 111 g was suspended in 1 liter of distilled water and boil it until it dissolves completely. ThenSterilize by autoclaving at 121C for 15 minutes as shown in Table.No.2.

Table.No.2: Composition of Mannitol salt agar (MSA)

Lab-lemon powder	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0
PH	7.5+-0.2 at 25C

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Antimicrobial susceptibility testing:

Antibiotic susceptibility pattern was determined by commonly used antibiotic by disc diffusion method different antibiotic are used Take colonies from an agar plate with a wire loop and transferred to the tube containing 4 to 5 ml of broth medium.The broth culture was then incubated at $37^{O}C$ for 2 to 8 hr.Take a dry nutrient agar plate and poured the broth containing colonies and was incubated for 15 mints at 37c.Placed sensitivity disk on the surface of nutrient agar at 24mm apart. After that plate was placed at $37^{O}C$ in an incubator.After 24 hours incubation the plates was examined.

Results:

A total of 650 samples were collected from infected patients visiting to MTI, KTH, Peshawarincluding samples urine, pus, wound, sputum, fluids. While total of 100 samples were collected of MRSA strains were obtained from pus (38.9%), followed by wounds (16.7%) sputum (5.6%), Fluids (11.1%), others (27.8%). as shown in Table.No.3 and Figure.N0.2.

Table.No.3: Distribution of different types of samples collected from infected patients

Different Samples	Male cases	Female cases	Total samples	Percentage
Pus	12	13	25	25.0
Wounds	5	5	10	10.0
Sputum	3	1	4	4.0
Fluids	8	6	14	14.0
Others	25	22	47	47.0
Total	53	47	100	100

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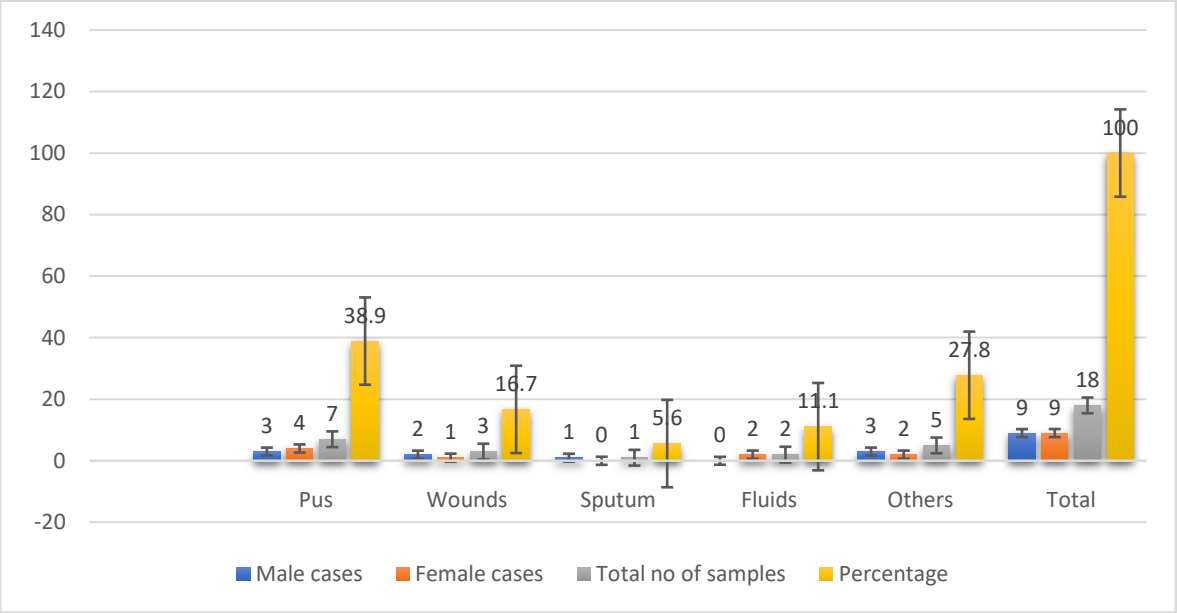

Figure.No.2: Distribution of different types of samples

Prevalence and Gender wise distribution of clinical isolates:

A total of 650 samples were collected among which 348 were collect from male patients and 302 were collect from female. Of the samples, 100(15.3%) isolates yielded the growth of *S. aureus*. Among positive isolates, 53 isolates were male and 47 were found in female patients. While in MSRA total 100 samples were collected 53 were male and 47 were females. Out 100 patients 18 are positive isolates the growth on MRSA. Among MRSA positive isolates, 9 isolates were male and 9 were found in female patients as shown in Table.N0.4 and Figure.No.3.

Table.No.4: Gender wise distribution of *S. aureus* positive isolates among infected patients (n=100).

Gender	**Total case**	**Total negative case**	**Total positive case**
Male	348	295	53
Female	302	255	47
Total	650	550	100

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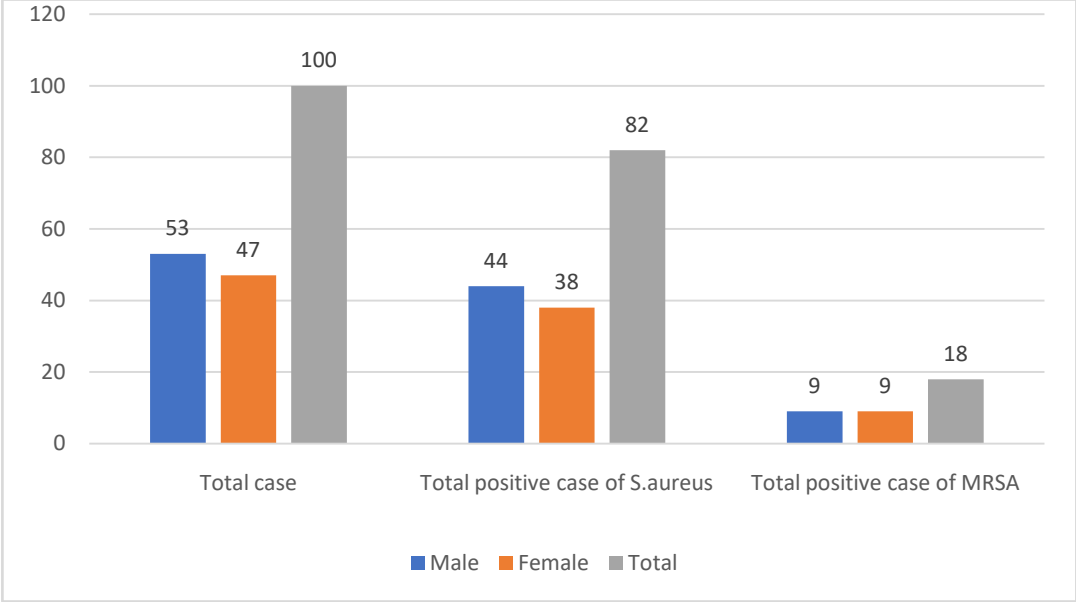

Figure.No.3: Gender wise distribution of MRSA from clinical isolates

Different age group:

S. aureus isolated from infected patients was distributed into different age groups. The highest prevalence of *S. aureus* was observed in the age group of 0-10 years (8.2%) followed by the age groups 11-20 years (4.4%), 21-40 years (7.1%), 41-60 years (4.6%) while the lowest prevalence of *S. aureus* was recorded in the age group of 61 and above years (3.0%). While MRSA isolated from infected patients was distributed into different age groups. The highest prevalence of MRSA was observed in the age group of 41-60 years (33.3%) followed by the age groups 0-10 years (27.8%), 11-20, 61 and above years (16.7%), while the lowest prevalence of MRSA was recorded in the age group of 21-40 years (5.6%) as shown in Table.No.5 andFigure.No.4.

Table.No.5: Different age group of patients among MRSA isolates

Different age group	Male cases	Female cases	Frequency	Percentage
0 – 10	3	2	5	27.8
11 – 20	3	0	3	16.7
21- 40	0	1	1	5.6

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41 – 60	2	4	6	33.3
61 and above	1	2	3	16.7
Total	9	9	18	100

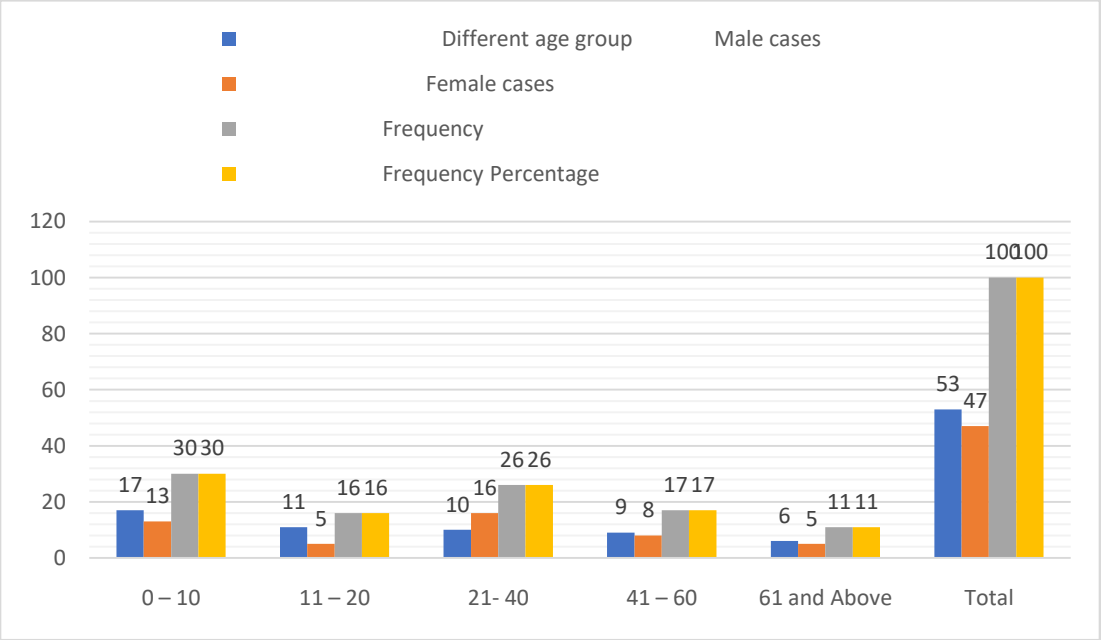

Figure.No.4: Different age group of patients among S. aureus isolates

Antibiogram of *staphylococcus aureus* in clinical isolates:

The present Research can screen 100 clinical samples isolates of staphylococcus aureus from different clinical samples. All the *S. aureus* isolates were tested against the selected antibiotics by using the standard protocol of CLSI guidelines. The antibiotic doxycycline showed highest sensitivity (98.0%) against the *S. aureus.* susceptibility to other antibiotics which were tested was as follows AK (97.0%), CTX (95.0%), LZD (93.0%), TEC (92.0%), V (92.0%), TGC (91.0%), CD (89.0%), FC (89.0%), CFC (85.0%), E(83.0%), FOX(82.0%), CRD(77.0%), CIP(68.0%), AMP(32.0%). AMC (29.0%) showed lowest susceptibility. While the highest resistivity pattern was observed in AMC (71.0%) followed by AMP (68.0%), CIP (32.0%), AK (3.0%), CRD (23.0%), DO (2.0%), FOX (18.0%), E (17.0%), CFC (15.0%), FC (11.0%), CD (11.0%), TGC (9.0%), TEC(8.0%), V(8.0%), CTX(5.0%). LZD (7.0%) showed the lowest resistivity against *S. aureus* as shown in Table.No.6. while other side 18 detected clinical isolates of MRSA from

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different clinical samples. All the MRSA isolates were tested against the selected antibiotics by using the standard protocol of CLSI guidelines. The antibiotic Vancomycin and Amikacin showed highest sensitivity (94.4%) against the MRSA. Sensitivity to other antibiotics which were tested was as follows TEC, DO and FC (88.9%), CFC (83.3%), CIP, CTX, TGC (72.2%), LZD, CRD,E,CD (66.7%), AMP(50%), AMC(38.9%), and FOX (0%) showed lowest susceptibility. While the highest resistivity pattern was observed in FOX (100%) followed by AMC (61.1%), AMP (50%), LZD, CRD, CD and E (33.3%), CTX, CIP, TGC (27.8%), CFC (16.7%), TZP, FC, DO(11.1%), AK and V(5.6%) showed the lowest resistivity against MRSA as shown in Figure.No.5.

Table.N0.6: Antibiogram of *staphylococcus aureus* in clinical isolates

Antibiotics	**Sensitive**	**Percentage**	**Resistant**	**Percentage**	***P* value**
AMC	29	29.0	71	71.0	246
AMP	32	32.0	68	68.0	655
TEC	92	92.0	8	8.0	575
CTX	95	95.0	5	5.0	748
FC	89	89.0	11	11.0	454
AK	97	97.0	3	3.0	630
DO	98	98.0	2	2.0	179
CIP	68	68.0	32	32.0	400
FOX	82	82.0	18	18.0	778
LZD	93	93.0	7	7.0	577
TGC	91	91.0	9	9.0	389
CRD	77	77.0	23	23.0	046
CD	89	89.0	11	11.0	241
CFC	85	85.0	15	15.0	274
E	83	83.0	17	17.0	032
V	92	92.0	8	8.0	575

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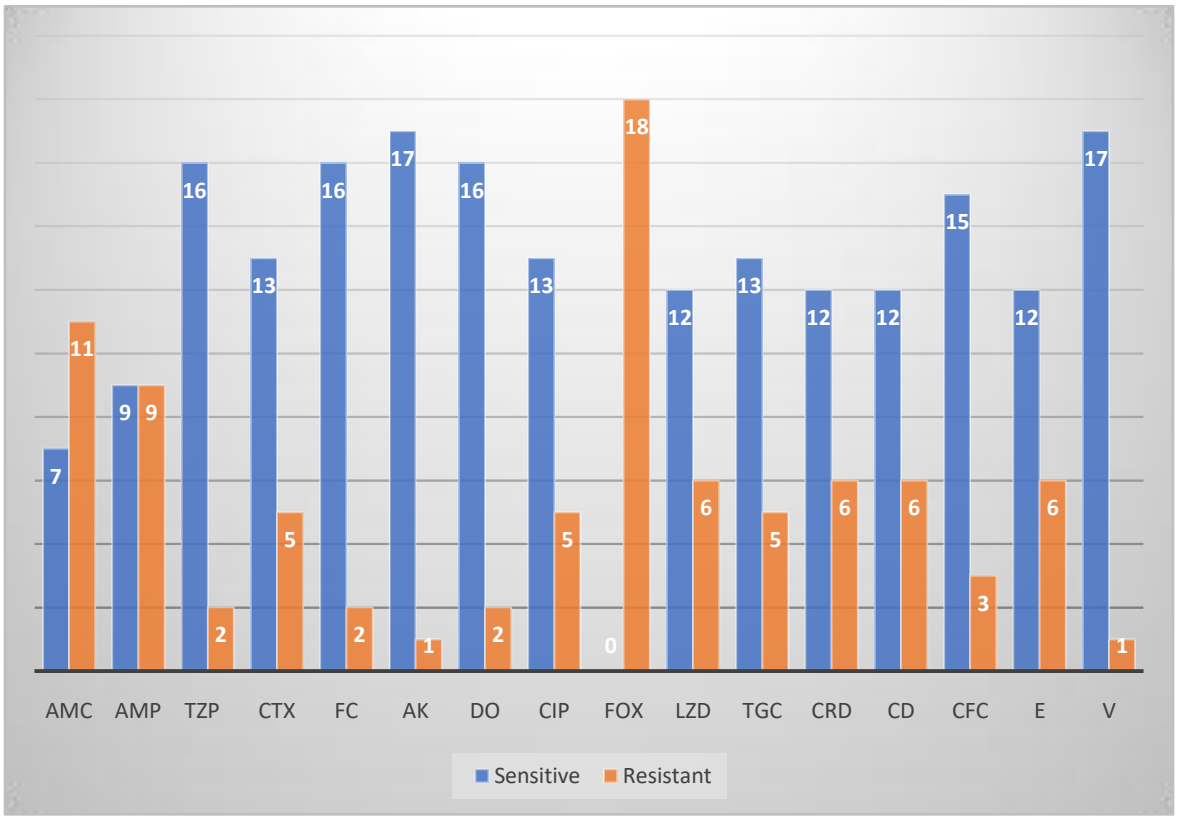

Figure.No.5: Antibiogram of MRSA in clinical isolates

Discussion

TheMRSA is a major issue faced by the medical sciences in the current era worldwide. The emergence of MRSA strains is an alarming situation for both hospitalized and non-hospitalized patients. Earlier studies have found that such infections have distinctive strain, virulence, and epidemiologic properties [19,20]. MRSA causes some serious infections, which is a great deal for the physicians, being associated with increase morbidity and mortality [21]. The results of the current study revealed that 18% isolates were found to be MRSA strains a total 100 isolates. Out of 18 MRSA isolates, 9 were from male and 9 from female cases. The high prevalence rate of 31% was observed in other study and in India the incidence rate of MRSA (31.8%) was reported as compared to this study [22,23]. The high prevalence of 46% was observed in other study than our findings [24]. In another study in Nagpur the rate of MRSA was 19.5% which is like this study [25]. Above studies clearly shows that prevalence of MRSA varies from one setting to other and our hospital being a newer one, the prevalence rate has been found at lower levels and

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it might increase with time. In the present study the prevalence rate of MRSA was higher in age group of 41-60 year (33.3%) and prevalence rate was lower in age group of 21-40 year (5.6%). Majority of MRSA strains were obtained from pus (38.9%), followed by wounds (16.7%) sputum (5.6%), fluids (11.1%), others (27.8%). However, no inference can be drawn from these values, due to low sample size. In the current study, Cefoxitin disc was used for the identification of MRSA i.e., recommended method for the detection of MRSA strains in clinical specimens [26]. MSSA isolates were show higher sensitivity to doxycycline (98.0%) followed by AK (97.0%), CTX (95.0%), LZD (93.0%), V (92.0%), AMP (32.0%), AMC (29.0%) showed lowest susceptibility. While the highest resistivity pattern was observed in AMC (71.0%) followed by AMP (68.0%), CIP (32.0%), AK (3.0%), CRD (23.0%), CTX (5.0%), LZD (7.0%) showed the lowest resistivity against S.aureus.MRSAisolates were show higher sensitivity to Vancomycin and Amikacin (94.4%) followed by Fusidic acid, doxycycline (88.9%), Cefachlor (83.3%), Ciprofloxacin (72.2%) and AMC (38.9%), FOX(0%) showed the lowest susceptibility . On the other hand MRSAshowed significant amount of resistant against FOX (100%) followed by AMC (61.1%), AMP (50%), LZD, CRD, CD and E (33.3%), CTX, CIP, TGC (27.8%), CFC(16.7%), TEC, FC, DO(11.1%), AK and V(5.6%) showed the lowest resistivity against MRS. The same results of MRSA isolates were observed in a study in which high resistance was recorded; Ciprofloxacin (73%), Ofloxacin (71%) and Erythromycin (67%). This is reliable with previous study completed at Ayub Medical Complex [27]. This may be due to the misuse of these antibiotics in daily practice at local hospitals. The results of our study are in accordance with a study carried out at Aga Khan University in 2009 which showed variable susceptibility pattern with high resistance rates to tetracycline (82%), clindamycin (79%), cotrimoxazole (59%), and rifampicin (50%). Resistance to chloramphenicol (10%) and Fusidic acid (9%) was low [28]. A study carried out at Lahore in 2009 showed that only 4% of MRSA isolates were sensitive to Fluoroquinoles, whereas 38% of isolates were found to be sensitive in our study [73].

Conclusion:

The current study concluded that 18% isolates were MRSA while the remaining isolates were MSSA. MRSA isolates were show higher sensitivity to Vancomycin and Amikacin (94.4%) followed by Fluidic acid, doxycycline (88.9%), Cefaclor (83.3%) while resistant against FOX (100%), AMC (61.1%), AMP (50%), CRD, CD and E (33.3%) and CTX, CIP (27.8%). Effective

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antimicrobial activity as well as cost effectiveness should be considered in drugs prescribed for MRSA infections. Oral dosing options for linezolid can allow earlier discharge of hospitalized patients and minimize the chances of MRSA emergence. Good hospital infection control measures prove to be the main stay against these infections because antibiotics can never be an effective alternative to good medical practice.

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