Prevalence & Biochemical characterization of Methicillin Resistant *Staphylococcus Aureus* in Pakistan, Rawalpindi.

Abdul Rafay*, Hira Arooj*, Muammad Noorul Amin**

University institute of biochemistry and biotechnology, Pir Mehr Ali Shah - Arid Agriculture University Rawalpindi*

Rawalpindi institute of Cardiology, Rawalpindi**

Abstract:

Present research study was conducted to reveal prevalence percentage of methicillin resistant *Staphylococcus aureus* (MRSA) and its biochemical characterization in Rawalpindi institute of cardiology (RIC) Rawalpindi. Five fifty six (556) samples were processed resulted in tentative isolation of 183 pathogenic strains from which only 10 were found to be the cause of MRSA cases, characterized on the basis of Catalase test, coagulase test, DNase and Kirby Bauer test. Such reported cases comprised approximately 2% of total samples collected and processed. Results of current study were found to be much lower than prevalence of methicillin resistant Staphylococcus associated infections in USA and UK. Whereas normal flora is also reported as no growth. The study also suggested that to avoid the spread of this Infectious diseases, physician and laboratory staff should follow proper quarantine measures while handling MRSA patients by Keeping them separate from rest of patients population under supervision as it is contagious. To further reduce the chances of dissemination of this disease, treatment must contain a proper combination of antibiotics to manage the MRSA infection as it can reside in healthy personal known as community associated (CA-MRSA).

Key words: methicillin resistant *staphylococcus aures* (MRSA), prevalence, contagious, dissemination

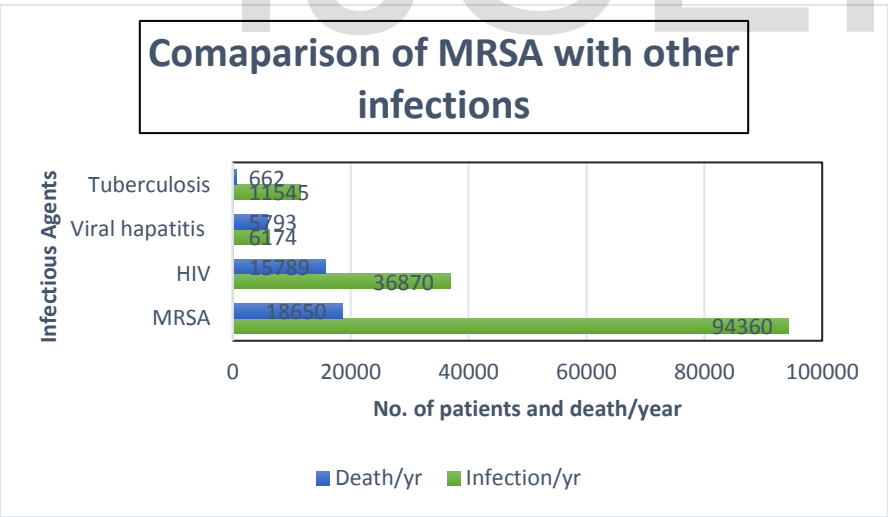
Introduction:

Staphylococcus Aureus is one of the most common name in clinical microbiology, responsible for worldwide morbidity and mortality [3]. *Staphylococcus Aureus* is a gram positive, catalase and coagulase positive Bacterium [4, 2, 12].it is aerobic, non-spore forming, non-motile and non-capsulated bacterium [12]. It is ubiquitous commensal bacterium on human skins and anterior nares/ upper respiratory tract [1]. It can cause serious diseases in human like blood Septicemia, endocarditis, osteomyelitis, carbuncles, pneumonia and soft tissues problems [2, 3, 13]. Toxin associated infections include toxic shock syndrome, food poisoning and exfoliative toxin causing scale skin problems in human [11,12, 13].

The patient who is already dealing with immunocompromised condition and admitted to hospital is more prone to MRSA infection. MRSA is mutant strain of *staphylococcus Aureus* genus that get the ability to deal with the beta lactam ring containing antibiotics. Bacteria produces an enzyme penicillinase that destroy the beta-lactam ring of antibiotic but MRSA does not adopt the same strategy. *Staphylococcus Aureus* get ability to mutate the penicillin binding proteins giving rise to MRSA. For the first antibiotic (penicillin) resistance was reported in 1941 and since that antibiotic resistance is on, it was happened just two years after clinical implementation of penicillin [14]. Penicillin resistant is palsmidic so it spreads quickly while methicillin resistance is chromosomic based therefore it spreads slowly, but it is there [14]. This type of infection is referred as health care associated MRSA (HA-MRSA) [15, 16]. While a weaken person with orophyrangial infection is discharged from a hospital he is source of MRSA for others. This type of infection spread is termed as community associated MRSA (CA-MRSA) [15, 16, 17]. In past people are infected with MRSA but now a days healthy people do have MRSA asymptomatically. Their colonization caused by dealing with already infected ones, cuts and wounds. It is a contagious infectious disease that is leading cause of community associated MRSA while HA-MRSA is much higher in prevalence. MRSA is major problem in nosocomial infections in modern terminology we can say health care associated infection (HAI) [16, 17]. Steps are being taken to eradicate the MRSA but orophyrangial infection not easy to tackle. Mupirocin is not enough to eradicate the MRSA, Rifampicin with fusidic acid in addition to topical mupirocin are applied eradicate the MRSA infection [20, 21, 22].

Staph infections are more common in people with a weakened immune system. This includes patients who are in hospitals and long-term care facilities for a long time, kidney dialysis (hemodialysis), Receive cancer treatment or medicines that weaken their immune system, inject illegal drugs, had surgery in the past year.

The revolution in antibiotics is a great hand in clinical microbiology to deal with the bacterial infection, but the same time this blessing being the curse to human in form of antibiotic resistant bacterium. In recent years antibiotic resistant microbes numbers getting higher. Antibiotic and environmental stress let the microbes revolutionize themselves. That's why pathogenic microbial prevalence is increasing day by day. MRSA (Methicillin Resistant Staph. Aureus) is one of the most primitive antibiotic resistant associated problem in clinical studies [14]. It's much more severe than a normal staphylococcal infection and may lead to death. Many of death cases reported throughout the globe [8, 9, 10] as given in following graph1.



Graph1: Infections per year in the United States. Deaths per year in the United States. (The graphs created by the author is in the public domain and thus free of any copyright restrictions) [8,9,10].

Our target was District Rawalpindi population visiting to Rawalpindi institute of cardiology for treatment.

MATERIALS & METHODS:

Survey and sampling:

For our final research problem we were collaborating with Rawalpindi institute of cardiology. In our 6 months research practice from November 2017 to April 2018, we go through 556 different specimen including pus, wound swab, catheter tip, pericardial fluid, urine, wound tissue, blood and sputum. Before the conduct of research we made sure sterility and safety. We collected all samples aseptically in a sterile manner. We used sterilized swabs, sterilized BHI bottles, disposable syringes and took care personal protection equipment (PPE). Sputum for AFB staining are not included in above given specimen. There were specific parameter for each patient including their specific ID number, gender, age, specimen and organism isolated [28].

Isolation:

Molar Hinton Agar (MHA) with specific contents (0.5% peptone, 0.3%yeast extract,1.5% agar,0.5% NaCl and distilled water with adjusted pH of 7.3), blood agar BA(nutrient agar + 5% sheep blood with ph 7.4), chocolate agar (heated blood agar at 80^{o}C, controlled RBC lysis), macConky agar(3g polypeptone, 17g peptone/gelysate, 10g lactose,1.5g bile salt mixture, 5g NaCl, 0.03g neutral red, 0.001g crystal violet,13.5g agar and 1l distilled water with ph 6.8) and nutrient broth (0.5% peptone, 0.3%yeast extract, 0.5% NaCl and distilled water). Streak the plates with inoculum or given specimen and incubate for 18-24hours at 37^{o}C aerobically and anaerobically in incubator. While blood is kept for 7 days in incubation for pathogenic isolation. After isolation incubated cultures proceeded for further confirmatory testing [14].

Morphological & Biochemical testing:

There are some characterized morphological parameters of each bacterial colony as colony shape, color, edges, size and specific shape (cocci, rods or vibrio). To visualize the single cell bacterium shape one must go for gram staining. Gram positive stained purple to blue while gram negative pink to red [1, 4, 23]. Following are the biochemical tests (catalase test, coagulase test, DNase test and KB testing) along brief procedures.

Catalase test: Catalase is an enzyme produced by the bacterium that break down H_2O_2 and releases bubbles of O_2 gas. Bubbles refer to positive for test while no bubble means negative, normally 3% H_2O_2 solution is used for testing [25].

Coagulase test: Coagulase is an enzyme that convert the (soluble) fibrinogen to (insoluble) fibrin in plasma of blood. Plasma taken in a test tube and a little mount of bacterial colony is mixed in that. Plasma clot in a few seconds. The test organism is positive for test otherwise negative. The test is performed as slide coagulase (SCT) or tube coagulase (TCT) method [4, 23]. Performance varies in different settings [6, 7].

DNase test: DNase is an enzyme that break down the DNA into small fragments. DNA containing medium in inoculated with test organism. After incubation (3ml) 1 N HCl is poured upon the colony if an opaque site is visualized it is DNase positive otherwise negative for the test [4, 24, 23].

KB-testing: also known as disc diffusion antibiotic sensitivity method or Kirby Bauer method. A disc with specific antibiotic concentration is placed on inoculated MHA plate to visualize the effect of antibiotics on susceptible organism. Each antibiotic has a standardized zone of inhibition [5, 15].

RESULTS & DISCUSSION:

All of collected samples are transported to microbiology laboratory for further proceedings immediately. We processed the samples by inoculating the sterilized blood agar (BA) and macconky agar (MA), Chocolate agar (CA). Incubated the samples at 37ºC for 18-24 hours aerobically. After incubation we took the plats to proceed further. Later we observe the colony morphology revealing shiny white to yellowish colonies on blood agar and white on chocolate agar. Hemolytic character (beta) on blood agar shows that staph Aureus is there. We prepared slides from the given incubated plate for microscopy. Clustered purple, round cocci confirm the staphylococcus specie characters [14].

For further confirmation we took a test tube with 3ml of 3% hydrogen peroxide and loop with mount of test organism. Dip the loop into test tube. Oxygen (O_2) bubbles confirm the catalase positive

staphylococcus specie (but S. aureus and & S. epidermidis both are positive for catalase test). We took the little mount of colony and mix that in blood plasma. The coagulated plasma confirm staphylococcus aureus. As it is compiled in given figure2. As staphylococcus epidermidis is negative for coagulase test. We have staphylococcus aureus but it is methicillin resistant or not is further confirmed by KB test.

For further confirmation of MRSA we used Kirbey-Bauer's method. We took MHA plate and staphylococcus aureus culture. Inoculate the plate with S.A (staphylococcus Aureus) and dispensed the given drug disk (Oxycillin, Methicillin and Vincomycin) on plate. Incubate the plate at 37^{o}C for 24hours aerobically. After incubation observe the size of inhibition zone. Each drug has specific drug concentration and zone of inhibition. For S.A we observed the vincomycin (VA) sensitive. For MRSA we observed the cifoxitin (FOX) sensitive (</= 22mm zone size), here we go with a normal staphylococcus Aureus but in case of a resistant FOX (>22 mm zone size) confirms the MRSA is there [27].

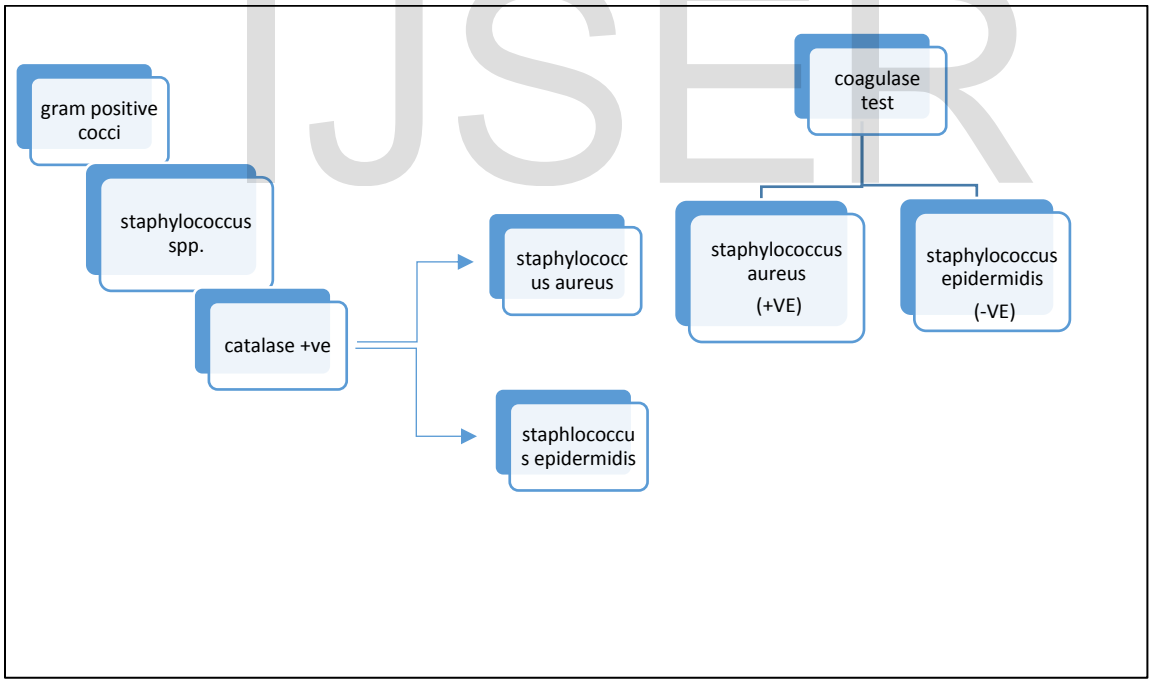
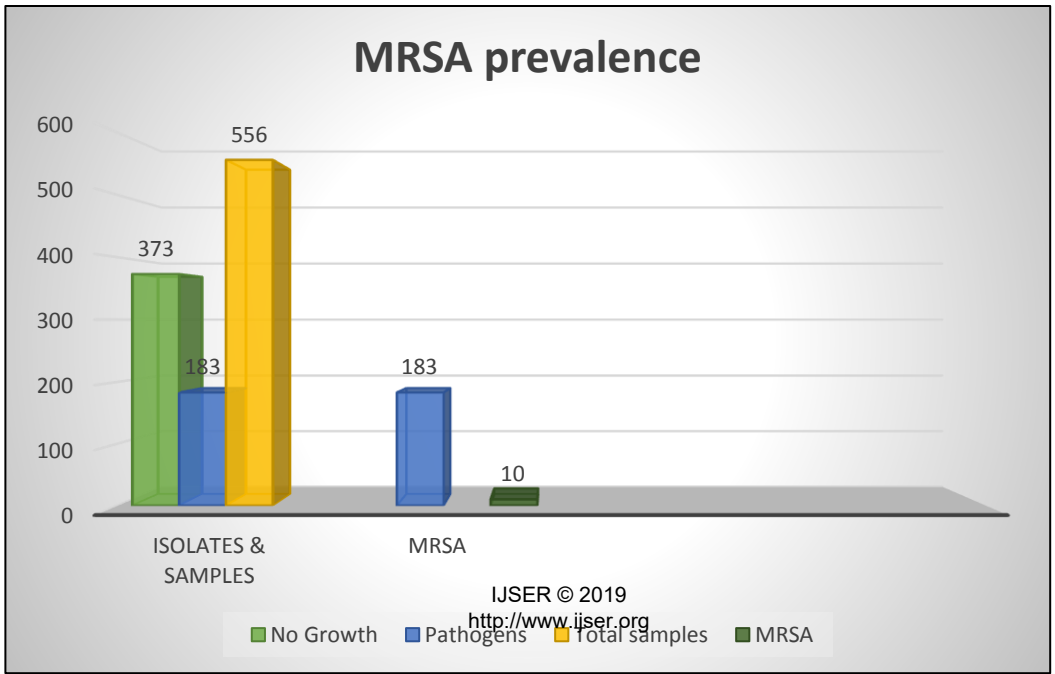


figure2: The above given flow diagram show how to confirm a staphylococcus Aureus from other species.

We have processed 556 samples in last 6 months and found only 183 positive for pathogenic growth while normal flora is also considered as no growth. Out of 183 pathogenic isolations we find out only 10 cases of MRSA, as it is shown in table2 & graph2. Out of 10 cases 6were females while 4 were male patients. We have an age group ranging from young kids of 6years to 87 year old patient. These ten of cases are given in table1. The given pie chart shows that 556 patients visited hospitals were supposed to be infected with pathogen while approximately 31% patients were actually infected and approximately 2% were infected by MRSA. While 67% of patient's samples were found with no growth and normal flora. According to statistical data almost 5.46% of actually infected were suffering from MRSA infection.

Age group of MRSA infected patients range from 27years to 71years. MRSA was isolated from pus, wound swab, blood, sputum and catheter tip. Mostly it comes from pus and catheter tips as it shown in given table1, as per given data females are bit more likely to get MRSA infection. It is not that much wide spread in Pakistan-Rawalpindi. There two potential reasons for this. First people are getting awareness about microbial infections and secondly hospital provide facility for appropriate microbial diagnosis. While in remote areas people lack this facility and physician mostly rely on broad spectrum antibiotics that can lead to a sudden outbreak of MRSA infection in rural areas. That may cause a high mortality rate in Pakistan's rural areas. To eradicate this problem national and international projects are working. WHO, PAK-TUK, USAID and Pakistan government trying to improve the health facility in urban and remote areas as well.



Graph 2: prevalence of MRSA among different isolates in dist. Rawalpindi.

Sr. No.	Age (years)	Sex	Specimen
1	60	F	Catheter tip
2	60	F	Blood
3	42	M	Catheter tip
4	27	F	Wound swab
5	59	M	Pus
6	54	F	Pus
7	52	M	Pus
8	61	F	Sputum
9	43	F	Pus
10	71	M	Wound swab

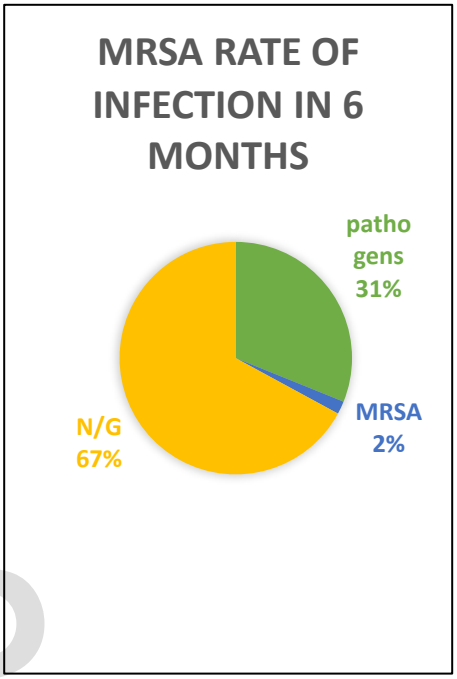


Table 1: MRSA associated with age gender & specimen. Pie chart reveals the emergence of MRSA in research time of 6 months that 2% of total isolation tests.

Month	Total samples	Positive (pathogens)	MRSA
November 2017	88	36	1
December 2017	50	15	2
January 2018	157	21	2
February 2018	73	34	0
March 2018	104	50	2
April 2018	87	27	3

Table 2: MRSA prevalence in dist. Rawalpindi

The all above given data in this article authentically confirms promising health outcomes in future. Hospitals facility, physician and microbiology laboratory lab staff must be trained by time. Public awareness seminars should be conducted regarding microbial infections and their effective treatments. Antibiotics should be administered in combination that better results can be obtained to manage the infection. Patients with MRSA should be kept separate from others. Environmental samples of hospital must be processed to confirm the sterile environment. Especially I.C.U, O.T, surgical instruments and patient's wards.

CONCLUSION:

Staphylococcus Aureus is a virulent clinical pathogen that is notorious for nosocomial infections. According to CDC and current trends in clinical studies S.A categorized in two types healthcare associated infection (HAI) and community associated infections (CAI). It has been leading to serious infections as it got resistant against antibiotics (FOX). MRSA is one of the most problematic strain of S.A now a days.. Microbes are still in progress as vincomycin resistant S.A and Oxycillin resistant S.A VRSA & ORSA are currently reported[15,18]. In Rawalpindi almost 5.46% cases of all pathogenic isolation were MRSA associated. Though it is very little as compare to prevalence rate in UK & USA [26]. But it is still there in aged and immunocompromised patients. Combination of ointments and oral drugs can effectively solve this problem. Better would be the healthcare facilities in Pakistan to takeover this problem.

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