Antibiotic resistant pattern of wound isolates of Staphylococcus aureus

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Abstract: Rapid resistance to most of the antibiotics has been problematic in therapy of Staphylococcal infection. Methicillin resistance Staphylococcus aureus was cured by vancomycin earlier. Later on vancomycin resistance S. aureus was emerged. The study was attempted to observe the antibiotic resistance pattern of wound infecting S. aureus. Altogether 55 S. aureus isolates were studied from 253 wound samples. Among them, 19 (34.54%) exhibited complete resistant to methicillin. In context of vancomycin, 4 (7.27%) were detected as resistant and 27(49.01%) appeared to be resistant to multiple drugs. Only 13.55% organism were susceptible to all antibiotics, 15.25% of them resistant to more than seven antibiotics. Majority (27.12%) were resistant up to three antibiotics. Multidrug resistance and Methicillin resistant isolates have statistically significant association ($\chi2 =7.025$; $p<0.05$). More than 85% isolates were resistant to β-lactams, 13% was intermediate to methicillin and oxacillin. Vancomycin, erythromycin, nitrofurantoin and streptomycin remained sensitive to more than 70%. Minimum inhibitory concentration of MRSA, ranged 8-256μg/ml and 1-32 μg/ml for oxacillin and vancomycin respectively with positive correlation ($r=0.85$) between both antibiotics.
Keywords: Methicillin, multi drug resistance, S. aureus, vancomycin, and wound infection

I. Introduction

Infectious disease, especially S. aureus infection was great burden in pre-antibiotic world as the mortality exceeded 80% and metastatic infection raised over 70% [1]. Scientists found antibiotics as miracle against most of the bacteria including S. aureus but their confidence was shaken by the emergence of resistance to multiple antibiotics by S. aureus. The bacteria have established itself as a causal factor for wide range of diseases. It is being more serious problem in children whose immune system is still not well developed and elderly people whose immune system is on the way to be worn out. Moreover, immuno-compromised patients have high chances of getting secondary infection by S. aureus. Prevalence of the bacteria is rapidly increasing in hospital acquired infection [2].

Penicillin, the first antibiotic was discovered in 1928. It was introduced clinically in early 1940s with initiation of antibiotic era [3]. Till then bacterial infections were terrifying. Fatality and mortality rate regarding bacterial infections declined significantly after initiation of antimicrobial therapy. It was positive aspect to strengthen medical sector. Medical personnel and scientists were stepping up confidently to conquer disease and germs then, they were suddenly stuck when antibiotic treated patients were still not recovering disease. Going through research, scientists obtained clue and it was the emergence of drug resistance microbes [1].

S. aureus has a unique character to resist the most antibiotics within a decade after it comes in use. They are well defined as promptly acquiring drug resistant. Initiating from penicillin till date it is resistant to linezolide which explains a kind of battle between antibiotics and bacteria [4]. Drug resistant is identified in different terms on the basis of antibiotics against which it shows resistance. Methicillin resistance S.aureus (MRSA), vancomycin resistance S.aureus (VRSA), Multi drug resistance (MDR) and extremely drug resistance (XDR) are some terms to describe its resistance pattern [5]. With regard to varying level of resistant predicted even in different clinical settings, the study was purposely begun to analyze and categorize antibiotic resistant S. aureus on the basis of resistance shown to key antimicrobials along with others.

II. Materials And Methods

The research was conducted in Department of microbiology and fermentation Technology, SHIATS, Allahabad. In consideration with high patient flow from both urban and surrounding rural areas, two tertiary care hospitals of Allahabad were chosen as study site. Pus from 253 patients were enrolled. After samples collected aseptically, they were transported to laboratory where isolation, identification and antibiotics sensitivity tests were performed.

All the 253 isolates were subjected to disc agar diffusion test (DAD) according to was Antibiotic disc diffusion Antibiotic resistance-pattern was studied by test [6]. Molten Mueller Hinton agar was poured in sterilized petri plates and swabbed with overnight cultures of isolated bacteria. Under aseptic condition, antibiotic discs were placed on the surface of inoculated plates, followed by overnight incubation at 37°C and zone of inhibition (mm) was measured. Minimum inhibitory concentration (MIC) was determined for methicillin non-susceptible strains obtained by DAD. About 5×10^4 cells in Mueller Hinton broth was treated with different concentrations of antibiotics and shaken for 16 hr at 37°C. The minimum concentration at which there was no visible turbidity was taken as the MIC of that antibiotic [7]. Isolates were classified as MDR on the basis of result obtained by DAD test basis of classification was referenced from Magiorakos et al. [5].

III.   Result

Altogether 253 clinical samples were examined and detected 55 isolates of S. aureus. Fig 1 describes the antibiotic resistance pattern of S. aureus associated with wound infection. Highest resistance was shown by penicillin (74.55%) followed by another β-lactam antibiotics, ampicillin (65.45%). Resistant isolates shows drastic decrease towards remaining antibiotics less than 40%. Vancomycin resulted highest susceptibility.

Stepping towards MRSA resistance pattern, All the isolates are entirely resistance to β- lactam antibiotics tested accept cephalosporin (42.11%). More than 40% of MRSA has shown resistance against gentamycin, tobramycin, amikacin, ciprofloxacin and tetracycline, similarly other antibiotics including vancomycin were only susceptible to less than 80% MRSA isolates. At the meantime, MSSA exhibited highly susceptible towards the drugs tested except penicillin, ampicillin and tetracycline. Though most antibacterial agents have shown comparatively high resistance in case of MRSA than that of MSSA, trimethoprim and tetracycline revealed no difference in resistance activity of both category of isolates. It is well explained by fig. 2 (MRSA) and fig. 3 (MSSA).

MIC of all the MRSA isolates for both oxacillin and vancomycin is graphically represented in fig 4. Statistical calculation of correlation coffecient showed the positive correlation (r=0.85) between them. Lowest MIC value for oxacillin and vancomycin was detected to be 8µg/ml and 1µg/ml whereas highest MIC value for them was 256µg/ml and 32µg/ml respectively. One of MRSA them obtained MIC 8µg/ml or vancomycin and categorized as VISA according to CLSI breakpoint.

Only 13.55% of all isolates were susceptible to all the antibiotics while 27.27% was resistant to more than 8 antibiotics (fig 5). Table 1 illustrates the association between MRSA and MDR. Table 1 illustrates the association between MRSA and MDR isolates ($\chi2_{cal}$ =7.025; p<0.05). Even if distribution of MDR is similar between MRSA and MSSA, Non MDR is significantly decreased in MRSA and prominent in MSSA.
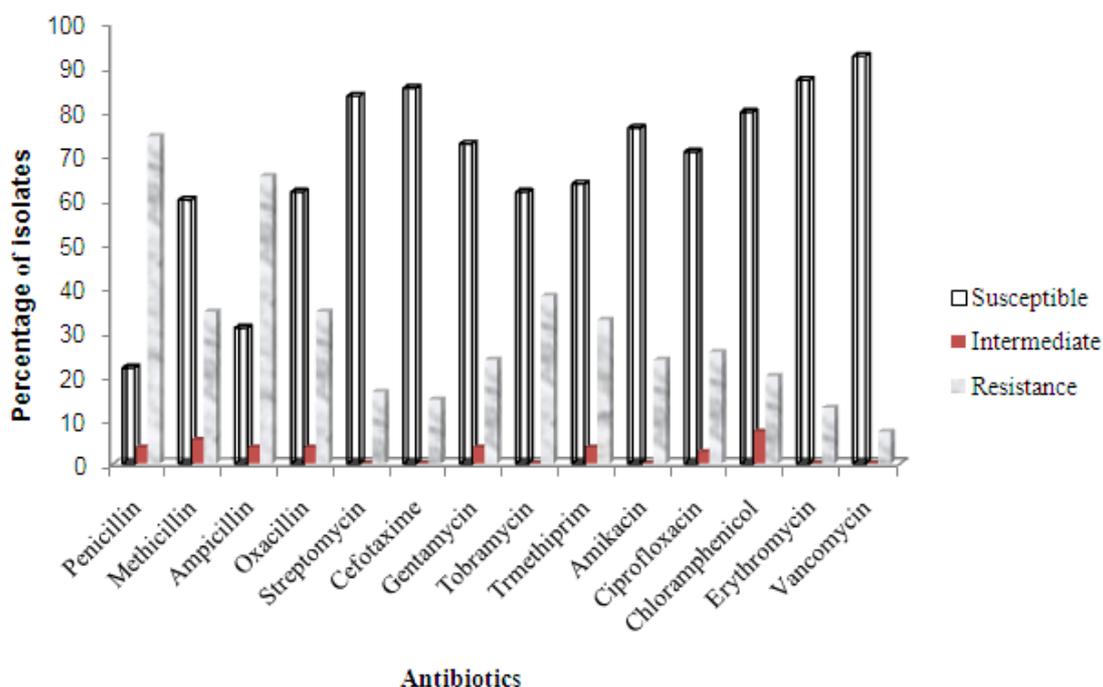
IV.   Figures And Tables

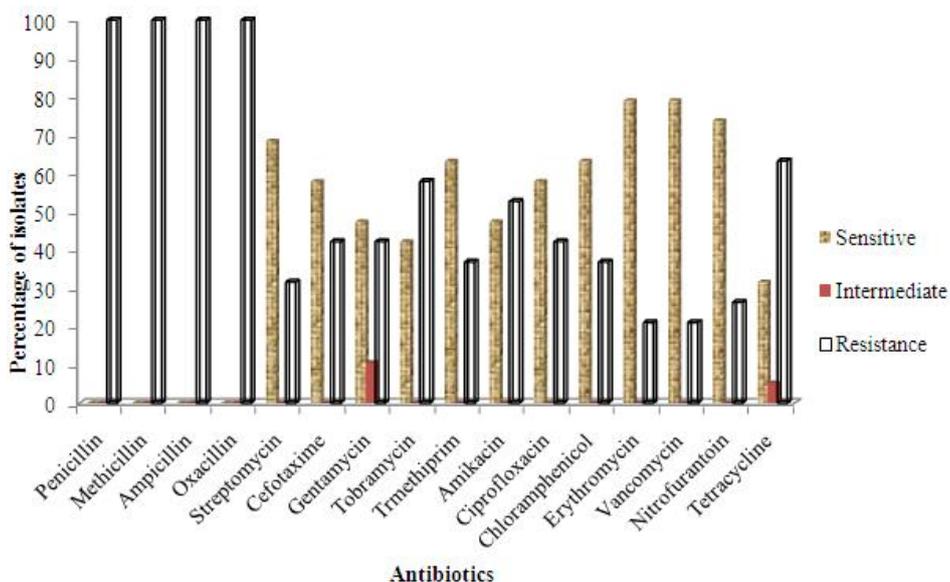**Fig 1:** Antibiotic sensitivity pattern shown by S. aureus isolates

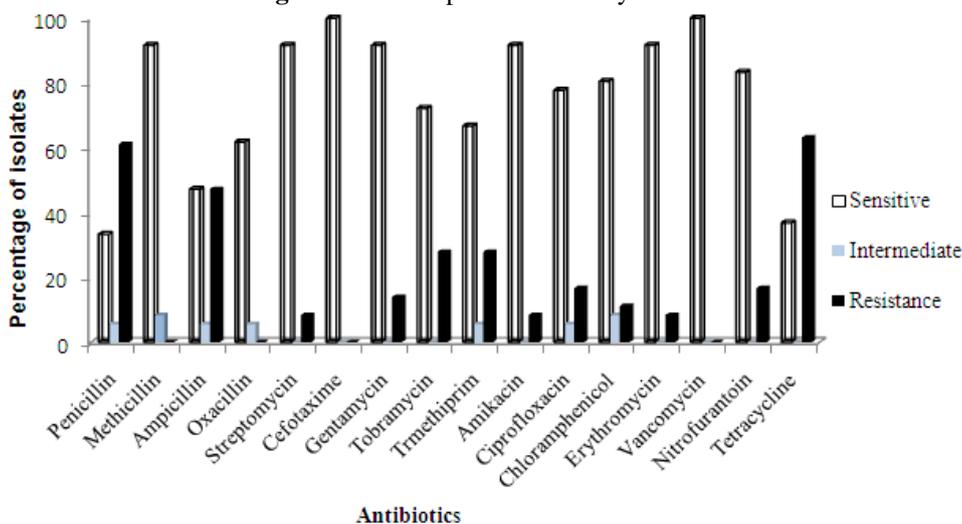**Fig 2:** Resistance pattern shown by MRSA

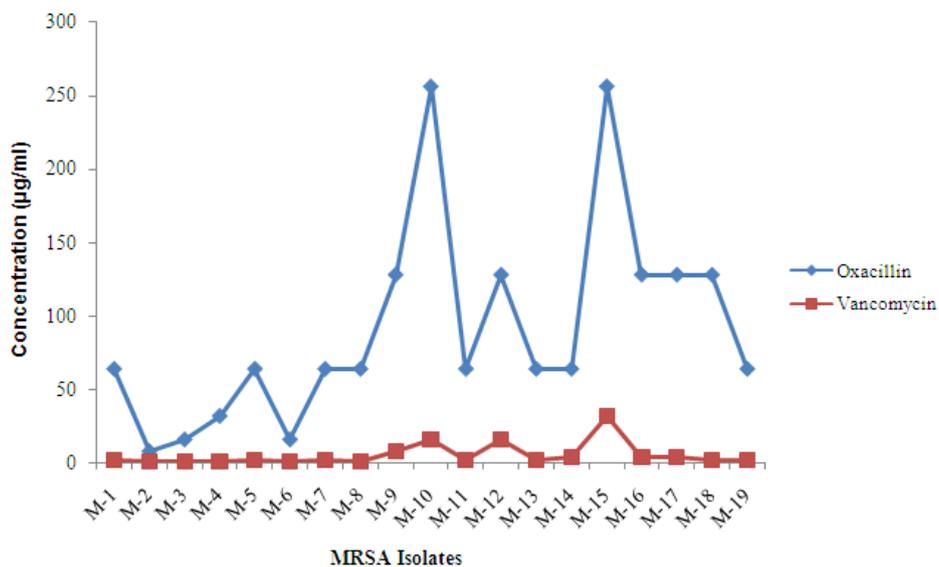**Fig 3:** Antibiotic sensitivity pattern of MSSA

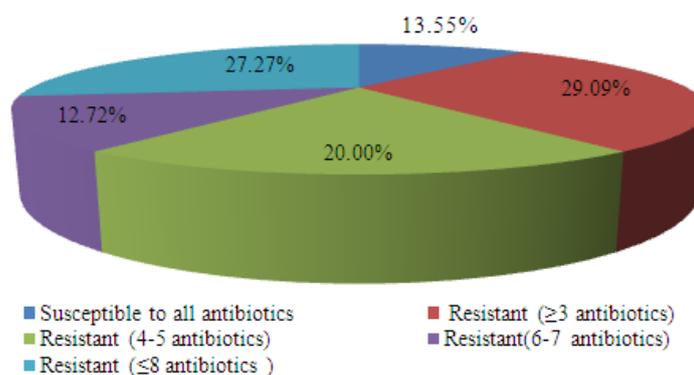**Fig 4:** Graph showing correlation between MIC of oxacillin and vancomycin

Fig 5: Pie-chart indicating the percentage of isolates resistant increasing number of antibiotics

Table 1: MRSA among MDR and non MDR isolates

Drug resistant type	MRSA	MSSA	Total
MDR	14	13	27 (49.01)
Non MDR	5	23	28 (50.90)
Total	19 (34.54)	36 (65.45)	55

Figures in parenthesis represent percentage
χ^2_{cal} =7.025 and χ^2_{tab} (1) = 3.84, 5% level of significance (P<0.05),
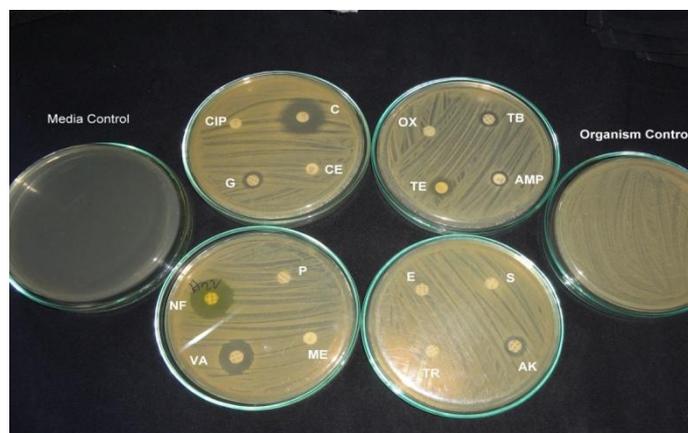
PLATES

Plate 1: Antibiotic susceptibility pattern of A-22 (M-10) isolate
P: Penicillin, ME: Methicillin, AMP: Ampicillin, CE: Cefotaxime, S: Streptomycin, OX: Oxacillin, G: Gentamycin, AK: Amikacin, TB: Tobramycin, TR: Trimethoprim, CIP: Ciprofloxacin, C: Chloramphenicol, E: Erythromycin, VA: Vancomycin, NF: Nitrofurantoin and TE: Tetracycline
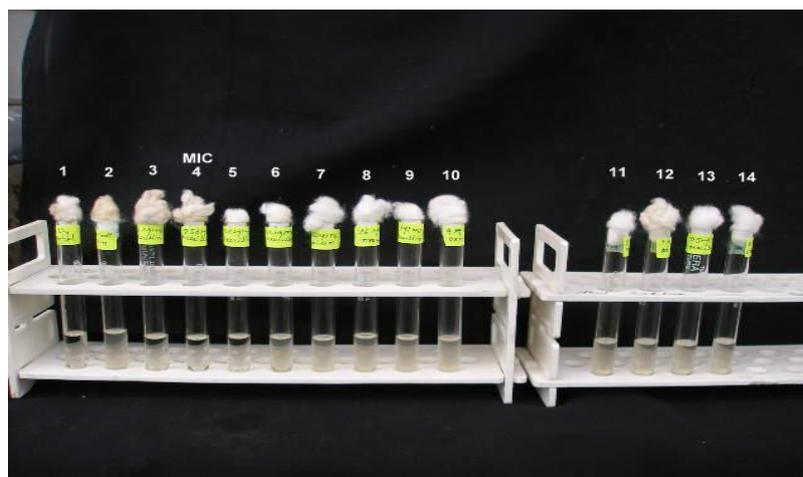

Plate 2: MIC determination of oxacillin for A-22 (M-10) isolate

1.Growth control, 2.Sterility control, **Oxacillin conc.:** 3. 512 µg/ml, 4. 256 µg/ml, 5.128 µg/ml, 6. 64 µg/ml, 7. 32µg/ml, 8. 16µg/ml, 9. 8µg/ml, 10. 4µg/ml, 11. 2µg/ml, 12. 1µg/ml, 13. 0.5 µg/ml and 14. 0.25µg/ml
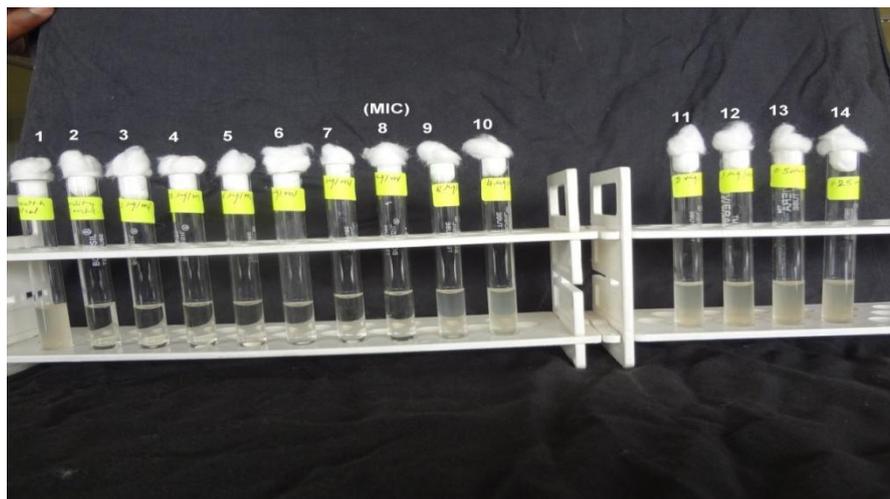

Plate 3: MIC determination of vancomycin for A-22 (M-10) isolate

1.Growth control, 2.Sterility control, **Oxacillin conc.:** 3. 512 µg/ml, 4. 256 µg/ml, 5.128 µg/ml, 6. 64 µg/ml, 7. 32µg/ml, 8. 16µg/ml, 9. 8µg/ml, 10. 4µg/ml, 11. 2µg/ml, 12. 1µg/ml, 13. 0.5 µg/ml and 14. 0.25µg/ml

V. Discussion

Antibiotic therapy changed infectious disease. Mortality rate due to bacterial sepsis declined dramatically after the initiation of antibiotic treatment. Till date, its importance in medical sector is incredible. However, emerging antibiotic resistant strains of bacteria is panicking the world. One of the common causal organisms for wound sepsis as well as nosocomial infection and presently spreading as community acquired S. aureus also belongs to rapid antibiotic resistance acquiring bacteria. Penicillin was first to be use against it and also first to be resisted. Still a couple of decades is left to complete a century since antibiotic period started and S. aureus has got resistance over more than major antibiotics (penicillin, ampicillin, methicillin, vancomycin and linezolide) consequently. These antibiotics were applied singly for Staphylococcal infection. Except these, the bacteria are resistant to many other antibacterial drugs of different classes.

Present data is no more different to result of several published and unpublished research within the duration of 10 years except a study done in Nigeria in 2007 that presented all the MDR strains of S. aureus to be methicillin and vancomycin sensitive though penicillin and ampicillin were remarkably high resistant (Yah, 2007). (Jacobus et al. 2007) carried research in 2005 in USA and found the 182 patients with cultures, prevalence of MRSA was 58%. His findings showed considerably high per cent MRSA (79.73%) resistant to vancomycin. Six VISA and two VRSA is mentioned by Tiwari and Sen (2006) [10]. MIC value for all of them exceeded 16µg/ml. Abdalla et al., 2010 from Egypt investigated 16% MRSA out of 1527 specimens [11]. Tsering et al. (2010) reported 38.14% MRSA among which 92.88% and 95.19% were resistant to penicillin and ampicillin respectively. 79.6% MRSA and 6.42% VRSA reported by Chakraborty et al., (2011) with MIC value of 4-64 µg/ml that of vancomycin. 30% MRSA and 26% MDR is revealed in study proceeded by Dhanalaxmi et al. (2012) [7,12,13]. None of S. aureus were resistant to vancomycin. INSAR group conducted research in 16 medical centers in 13different places during the period of 2008-2009 and investigated average 42% and 40% MRSA in 2008 and 2009 respectively [14]. Higher frequency of MRSA was found to be resistance to antibiotics than that of MSSA in present study which also resemble with that of multicentered research proceeded by INSAR group. This result is supportive for the positive association between MRSA and MDR obtained presently.

Surveillance of 30 months in an Indian teaching hospital during 2010 to 2012 has shown 20-81% MRSA among S. aureus detected from various clinical samples and 20% VRSA among total MRSA. High resistance rate was for reported other individual antibiotics of β-lactams, carbepenems, imipenems aminogycosides, macrolides, floroquinolones, sulfonamides and tetracycline [15]. On MIC determination, 9 isolates for both methicillin and vancomycin exceeded 256µg/ml each.

Several mechanisms of resistance have been understood. Enzymatic degradation of chemotherapeutic agent, mutation in concerned gene, target alteration and reduced intracellular concentration of drug are the major mechanisms. No doubt, most of the resistance due to genetic basis are mediated through the movement of plasmid either it is penicillin, methicillin or vancomycin [1]. β-lactamase responsible for cleavage of β-lactam ring is produced by more than 70% of staphylococci and was the mere cause for resistance of β-lactam

antibiotics prior to introduction of β-lactamase stable agents [3]. It left no choice other than entry of novel penicillinase stable β-lactams. Then after methicillin replaced penicillin, unfortunately within a couple of years resistance strains appeared. Altered target site in penicillin binding protein was forwarded as reason [16].

All the novel antibiotics lasted shortly against S. aureus except vancomycin which remained stable in staphylococcal treatment for many years until a first case of VISA identified in Japan in 1997 followed by two more in USA. This evidence was slowly getting strong as more VISA was reported from time to time in different countries. Finally in 2002, VRSA was isolated in Michigan hospital from diabetic patient [17]. Since then, many countries around the globe published the reports of finding VRSA with elevated MIC range [19]. S. aureus spread out epidemiologically in critical care units of some hospitals of USA. Epidemiological evidences clearly revealed genetic transfer of increased level of vancomycin resistance from Enterococcus. One common factor was pointed out all of above cases i.e. inter-species and intra-species horizontal transfer of plasmid carrying resistance genes which reasons the fact that once the novel resistant mechanism detected, within short period it is wide spread [17].

Few cases rendering decreased permeability of cell wall or pumping the drugs out of cell wall. both generalized and specific efflux pumps is in existence within the bacterial cell. Specific type causes for extruding single antimicrobial agents of same class whereas generalized type are incapable of removing more than one classes forwarding to MDR cases [19]. Irrational use of chemotherapeutic agents with inconsideration of recommended duration is common in public and rural clinical setting. They are unknown of consequences leaded by their act. Nevertheless, different classes of antibiotics are used in treatment of staphylococcal infection; key antimicrobials are same all over the world. Dose and duration of complete course, time interval between every intake have also significant role. Variation in all theses unseen but key factors account for varying level of resistance to variable classes of antimicrobials in different parts of globe endangering therapeutic [13,19].

VI. Conclusion

Among various pathogen S. aureus is also one major agent in causing wound infection. Variable frequency of resistance against key antimicrobials is revealed globally relying on the continental variation, county and regions within the same country even the hospitals of same places. Moreover scenario of successively emerging resistance in these superbugs has created tenacity to every related domain. MDR S. aureus is spiraling to extremity. One major supportive factor is increase in MRSA. Prognosis is getting tough to cope this notorious pathogen. Periodic surveillance has significant importance in finding clue for further movement.

Acknowledgement

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