ORIGINAL ARTICLE

Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* Isolated from PNS Shifa Hospital, Karachi, Pakistan.

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Abstract

Objective
To determine antimicrobial susceptibility pattern of *Staphylococcus aureus* in a tertiary care hospital, Karachi.

Study design
Descriptive cross sectional.

Place and Duration of study
The study was carried out in Department of Microbiology, PNS Shifa hospital, Karachi from June 2014 to May 2016.

Materials and Methods
A total of 2240 samples are selected for the study among which 1132 samples revealed the growth of different microorganisms. Samples of pus, blood, body fluids, and sputum and wound swabs are included and different biochemical reactions are performed in order to identify *Staphylococcus aureus* isolates. The antimicrobial susceptibility of these isolates are performed by disk diffusion method using cefoxitin (30µg) and different antibiotics are tested against methicillin resistant and sensitive staphylococcus isolates as outlined by Clinical Laboratory Standard International Guidelines.

Results
Among different samples, total 592 samples showed the growth of *Staphylococcus aureus*. Among them n=377 (64%) isolates were methicillin resistant *Staphylococcus aureus*, and n=215 isolates were methicillin sensitive (36%). Out of 377 isolates of methicillin resistant *staphylococcus aureus* (MRSA) from different clinical samples, 79% (n=298) isolates are from pus samples. We have also isolated 215 MSSA, 82% (n=176) are isolated from pus. Out of 377 isolates of MRSA, 41% (n=153) are susceptible to tigecycline, 15% (n=57) susceptible to erythromycin, 46% (n=172) sensitive to clindamycin, 28% (n=106) sensitive to cotrimoxazole, 66% (n=250) sensitive to doxycycline, 72% (n=272) sensitive to chloramphenicol, and 17% (n=65) sensitive to ciprofloxacin. Among 215 isolates of *staphylococcus aureus (*methicillin sensitive), 50% (n=108) of isolates were sensitive to tigecycline, 41% (n=88) were sensitive to erythromycin, 55% (n=118) were sensitive to clindamycin, 53% (n=113) were sensitive to cotrimoxazole, 74% (n=160) were sensitive to doxycycline, 71% (n=152) were sensitive to chloramphenicol, 58% (n=125) were sensitive to ciprofloxacin. All strains of *Staphylococcus aureus* (methicillin resistant and sensitive) are found susceptible to vancomycin and linezolid, 100% (n=592).

Conclusion
The antibiotic susceptibility pattern obtained from the above study showed a lot of antimicrobial choices available for *staphylococcus aureus*, so the use of Vancomycin and linezolid should be the last resort for treating such infections. It also draws attention towards increasing resistance among the widely used antibiotic such as ciprofloxacin so its overuse should be discouraged.

Key words
Antimicrobial susceptibility, Methicillin resistant *Staphylococcus aureus*, *Staphylococcus aureus.*

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Introduction
Staphylococcus aureus is one of the most prevalent and shared pathogen involved in human infections.[1] It causes a broad range of infections which includes skin and soft tissue infections, pneumonia, infective endocarditis leading to septicemia.[1] It has been implicated as one of the most common organisms involved in post-operative wound infections.[2] Nasal carriage among health care workers is the main source of nosocomial infections. Colonization rate of healthcare personals is greater, most likely due to increased exposure.[3]

In recent past there has been an alarming increase in methicillin resistant *Staphylococcus aureus* (MRSA) in hospital settings.[4] The rise in hospital admission for CAP (community acquired pneumonia), VAP (ventilator associated pneumonia) and surgical site infections are linked with increased prevalence of MRSA.[5]

Before the discovery of penicillin, staphylococcal septicemia was a major threat.[1] Within a short span bacteria developed resistance to penicillin and therefore rendered ineffective.[2,7,8] In 1960s beta lactamase penicillin's emerged as revolutionary

drugs, but soon they failed after the emergence of methicillin resistant *Staphylococcus aureus* (MRSA). Against MRSA vancomycin is the suitable antibiotic and the drug of choice.[9,10] The present predicament is the emergence of vancomycin resistant strains of *staphylococcus aureus*, and thus the treatment options are also available for such resistant strains.[11,12]

The rationale of our study is to detect the antimicrobial susceptibility pattern of *Staphylococcus aureus* both methicillin resistant and sensitive isolated in a tertiary care hospital, Karachi and to limit the unnecessary usage of broad spectrum antibiotics.

Materials and Methods

This study was conducted in the Department of Microbiology, PNS Shifa Hospital, Karachi. The study protocol was approved by institutional ethical and research committee. The duration of study is from June 2014 to May 2016. A total of 2240 samples received for culture and sensitivity among which 1123 samples were positive for the growth of microorganisms. Around 592 samples showed the growth of *Staphylococcus aureus*. These isolates further studied for beta-lactamase production, and 377 isolates found to be methicillin resistant.

Samples were inoculated on blood and MacConkey agar plates and incubated at 37ºC aerobically for 24 to 48 hours. On the basis of beta-hemolytic colonies sample selection made with yellowish pigment found on blood agar. Further test were performed for confirmation of *Staphylococcus aureus*. These test include catalase test, slide coagulase test and tube coagulase test, DNAse test.[12]

Antimicrobial susceptibility testing was carried out by Kirby –baeur disc diffusion method as outlined by Clinical Laboratory Standard International Guidelines.[13]

Methicillin resistance was confirmed by Kirby-baeur disc diffusion method using Mueller Hinton agar plate, supplemented with 7% Nacl and 30µg cefoxitin disc (OXOID). An isolate observed methicillin resistant if zone of inhibition of 30µg cefoxitin is ≤21mm.Results were evaluated on the basis of criteria found in CLSI and the ATCC control used in this procedure is *Staphylococcus aureus* (ATCC29213).[13]

The antibiotics tested for susceptibility includes cefoxitin (30µg), gentamycin (10µg), amikacin (30µg) erythromycin (15µg), clindamycin (2µg), trimethoprim-sulfamethoxazole (1.25µg), chloramphenicol (30µg), doxycycline (30µg), ciprofloxacin (5µg) and linezolid (30µg).(OXOID).

Data analysis was done using IBM SPSS and regressions were applied to find any association between independent and dependent variables.

Results

Among different samples, total 592 samples showed the growth of *Staphylococcus aureus*. Among them n=377 (64%) isolates were methicillin resistant isolates, and n=215 isolates were methicillin sensitive (36%). Out of 377 isolates of methicillin resistant *Staphylococcus aureus* (MRSA) n=298 (79%) isolates are from pus samples, n=56 (15%) from blood, n=11 (3%) from tip, n=4 (1%) from cerebrospinal fluid and n=7 (2%) from pleural and ascitic fluid as shown in Figure 1. Out of 215 MSSA isolates of *staphylococcus aureus* n=176 (82%) are from pus samples, n=21 (10%) are from wound swab, n=15 (7%) are from blood and n=2 (1%) from tip as shown in Figure 1.

Among 377 isolates of MRSA,41% (n=153) are susceptible to tigecycline,15% (n=57) susceptible to erythromycin, 46% (n=172) sensitive to clindamycin, 28% (n=106)sensitive to

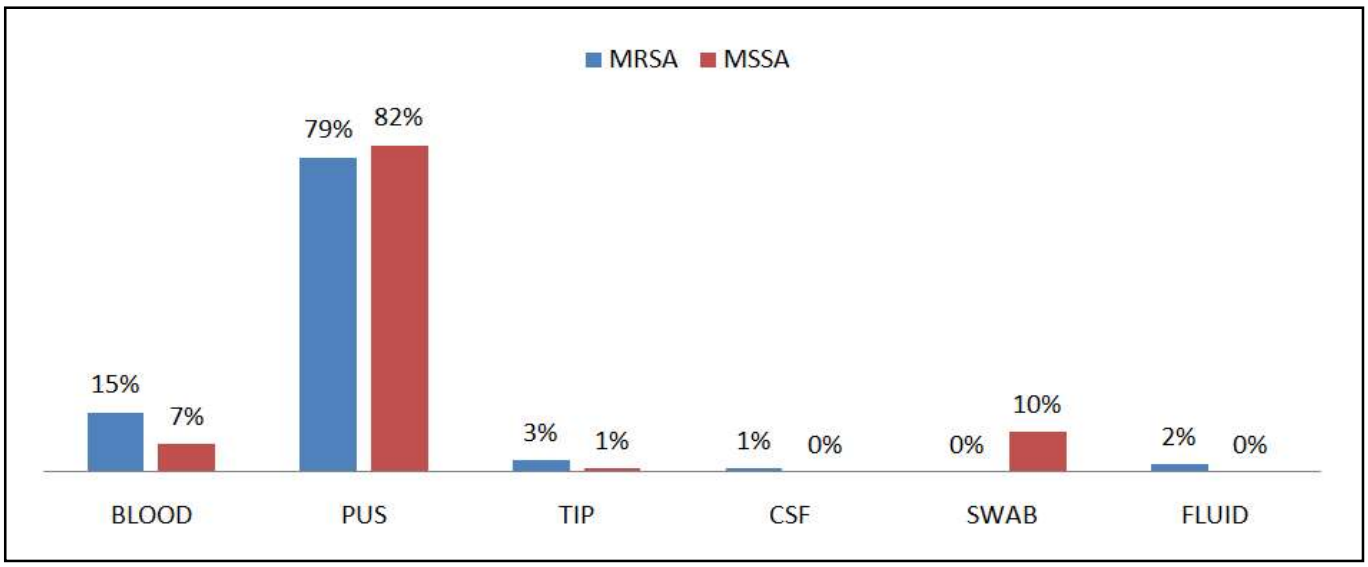

Figure 1: Percentage of MRSA (n=377) and MSSA(n=215) isolates

cotrimoxazole, 66% (n=250) sensitive to doxycycline,72% (n=272) sensitive to chloramphenicol, 42% (n=158) sensitive to gentamicin, 37% (n=139) sensitive to amikacin and 17% (n=65) sensitive to ciprofloxacin. Antibiogram is shown in Figure 2.

Among 215 isolates of MSSA, all are sensitive to oxacillin, amoxicillin and cephradine,100% (n=215), 50% (n=108) of isolates were sensitive to tigecycline, 41% (n=88) were sensitive to erythromycin, 55% (n=118) were sensitive to clindamycin,53% (n=113) were sensitive to cotrimoxazole, 74% (n=160) were sensitive to doxycycline, 71% (n=152) were sensitive to chloramphenicol, 90% (n=193) were sensitive to gentamicin, 81% (n=174) were sensitive to amikacin, 58% (n=125) were sensitive to ciprofloxacin as shown in Figure 3.

All strains of MSSA and MRSA are found susceptible to Vancomycin and linezolid (100%).

Discussion

One of the most common causes of nosocomial infections in the current research setting is methicillin resistant *Staphylococcus aureus* (MRSA). Its increasing prevalence is a major threat to our healthcare system as it not only increases the hospital admissions but also linked with increased mortality rates.[14]

The prevalence of MRSA and its antibiotic susceptibility pattern is a substantial aid for a clinician to treat such infections.[15] The prevalence of MRSA found in the above study is 63.38%, which is in agreement with the Iranian study showing the prevalence around 60%.[12]

The prevalence of MRSA in a recent study conducted in Peshawar, Pakistan was 36%, which is less than found in our study.[16] This difference is due to the change in the environmental conditions, seasonal variations, difference in blood culture system, and type of patient population.[16]

The progressive increase in the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) and its association with non-judicical use of antimicrobials is a matter of concern not only for clinicians but also for microbiologist and constitute a major global risk. The frequency of MRSA in our study is highest in pus (79%), followed by blood (15%),while the frequency of MSSA is 82% in pus and 10% in wound swab. This is comparable to a study conducted in Ethiopia, where incidence

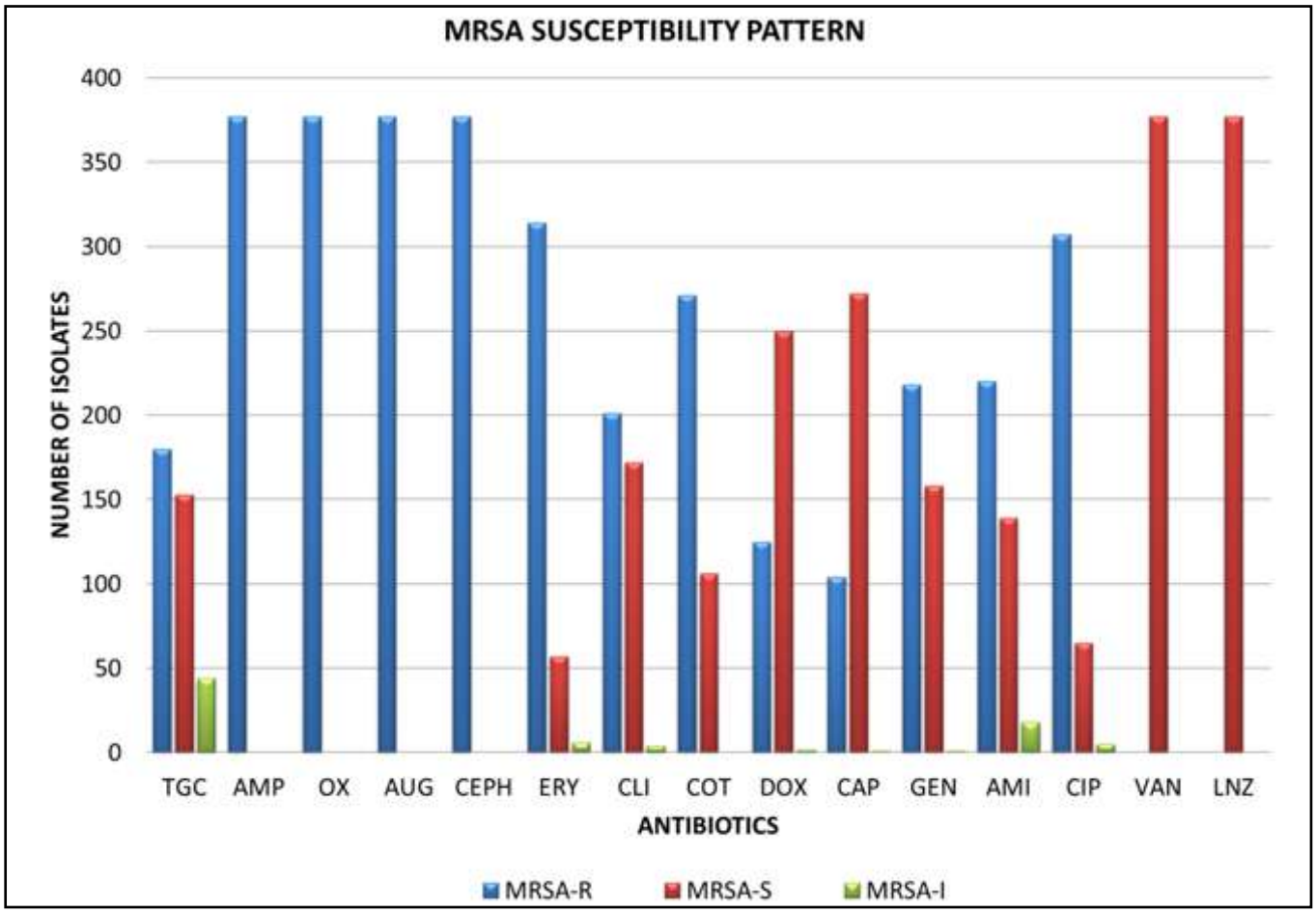

Figure 2: Antibiogram of MRSA isolates (n=377).

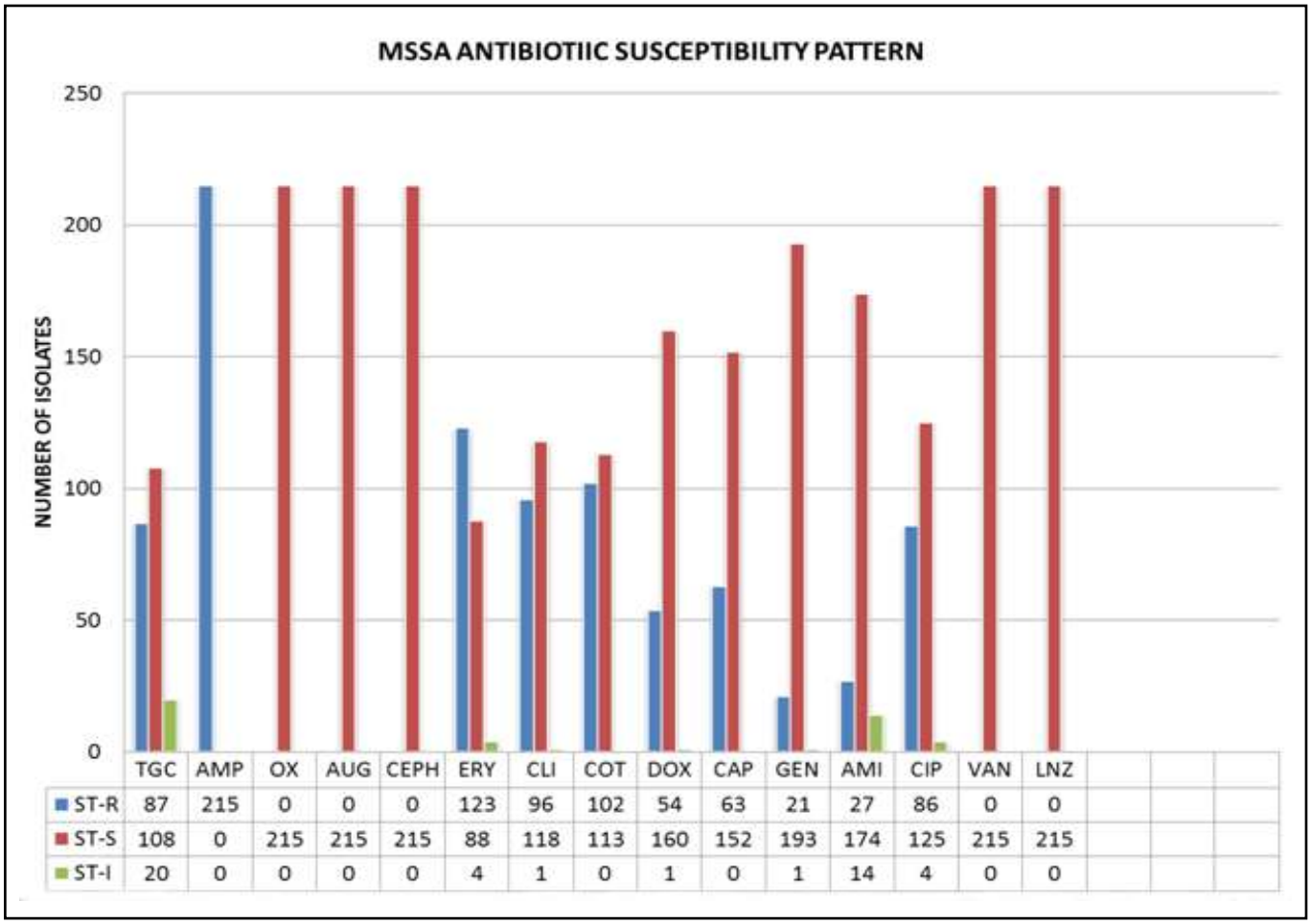

Figure 3: Antibiogram of MSSA isolates (n=215).

of *staphylococcus aureus* both methicillin resistant and sensitive is highest in pus (55%).[17] The result is also in concordance with Indian studies reporting highest prevalence of MRSA in pus followed by blood.[19,20]

Among antibiotics, majority of *staphylococcusaureus*, both methicillin resistant and methicillin sensitive is susceptible to chloramphenicol, (72% for MRSA) and (71% for MSSA). Antibiotic susceptibility against doxycycline is also high as comparable to other antimicrobials. All isolates are 100 percent sensitive to Vancomycin and linezolid. Similar results are also obtained in a study carried out in Northern areas of Jordan in 2015.[18]

Vancomycin and linezolid are the only antimicrobials showing 100% sensitivity to all staphylococcus isolates, which is also observed in studies carried out in different regions of Asia.[1,2] Same pattern of sensitivity against Vancomycin was also found in a recent study carried out in Namibia.[23]

One of the most striking point concluded from the above data is the increasing resistance of ciprofloxacin (17% sensitive) for MRSA. The emergence of resistance of ciprofloxacin (17% sensitive) is a characteristic feature of the current research. This may presumably be related to the inappropriate antibiotic use in hospital setup and outpatient departments.[22]

The reason behind this resistance is mainly the overuse of this antibiotic in hospital setup and outpatient departments in our country, same pattern of resistance is also observed in a recent study conducted in Iraq.[21,22] A recent study carried out in Namibia showed different susceptibility pattern of ciprofloxacin.[23]

This variation may be related to the change in the targeted population, culture techniques and different preferences of clinicians to choose the appropriate antimicrobials.

Antibiotic resistance is a major global threat, and one of the most unsolved problem in public health. This is a point of concern for clinicians, microbiologist and pharmacist. It leads to the efforts of pharmaceutical companies in manufacturing new antimicrobials effective against the resistant microorganisms. After a certain period of time, those antimicrobials again become ineffective either due to their overuse or the emergence of new

resistance mechanisms developed by the pathogens. These organisms continuously acquire new antibiotic resistance and virulent factors.

The limitations of this study are that all the samples included are obtained after admission and from outpatient department who already may have been taking antibiotics. Detailed clinical evaluation was done in order to correlate the results and to obtain their clinical significance.

Conclusion

The antimicrobial susceptibility pattern obtained from the above study displayed the increasing resistance in overly prescribed drugs especially ciprofloxacin against *staphylococcus aureus*. Attention should be given to restrict the non-judicial use of antimicrobials such as vancomycin and linezolid, used among admitted patients.

Conflict of interests

We have no conflict of interest.

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