Evaluation of antibiotic resistance pattern in clinical isolates of *Staphylococcus aureus*

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Abstract: *Staphylococcus aureus* is a common skin colonizer as well as opportunistic pathogen causing serious diseases including bacteremia, endocarditis and a number of different infections. It has a unique ability to swiftly respond and develop resistance for every other antibiotic introduced against it. The prevalence of antibiotic resistant strains of *S. aureus* is increasing on an alarming rate, which not only restrains the treatment options but the economic deprivation sustained due to infections of this superbug are incomputable. In our study, antimicrobial resistance patterns for 13 different antibiotics were evaluated in non-duplicate isolates of MSSA and MRSA isolated from different clinical samples (i.e. urine, pus, HVS, blood, tissue, wound and ear swabs). Most cultures were identified as multi-drug resistant (MDR). The highest resistance was recorded against ampicillin and erythromycin (88% each), while resistances against oxacillin, fosfomycin, cefoxitin and ciprofloxacin were also worrisome. No strain was sensitive to all antibiotics. Resistance levels of MSSA against ampicillin, erythromycin, fosfomycin and fusidic acid were also high. Least level of resistance was observed in case of vancomycin. Only 12% isolates were resistant to vancomycin, among which 24 were MRSA and 6 was MSSA.

Keywords: *Staphylococcus aureus*, MDR, MRSA, MSSA, disc diffusion.

INTRODUCTION

Staphylococcus aureus is a member of natural human flora, present on skin surface and mucous membranes (particularly of nasal area), which can cause severe infections as soon as it gets a chance to penetrate the internal tissues or bloodstream (Taylor and Unakal, 2018; Weiner *et al*., 2016).

Staphylococcus aureus has an anomalous ability to quickly develop resistance against every other antibiotic. The mechanisms for resistance are numerous including inactivation of antibiotics by enzymes, targ*et al*teration with decreased affinity for the antibiotics, antibiotic trapping, efflux pumps etc. (Benveniste and Davies, 1973; Piso *et al*., 2017). On the basis of resistance development, there are two types of *Staphylococcus aureus.* Those that are resistant to β-lactam antibiotics (a group of broad spectrum antibiotics including some penam-penicillin derivative such as methicillin and oxacillin, and cephalosporins such as cepham are termed as are termed as MRSA-Methicillin resistant Staphylococcus aureus, whereas those that are susceptible to these are termed MSSA-Methicillin susceptible *Staphylococcus aureus* (Gurusami, *et al*. 2013).

Antimicrobial resistance among pathogens is becoming a life threatening problem world-wide. Excessive usage of antibiotics has caused 23,000 mortality cases due to development of antibiotic resistant bacterial infections (CDC, 2013). Antibiotic resistant infections can occur anywhere in the community, but the rate of nosocomial infections is even higher. Resistance in bacteria is a serious matter because as bacteria evolve and form ways to nullify the antibiotics, the antibiotics become ineffective, letting these deleterious organisms to survive in multiple environments (Margonis *et al*., 2018; Wang *et al*., 2016).

Now the world is entering a 'post-antibiotic' era. In Nigeria β-lactam antibiotics don't work on 88% of *S. aureus* infections. According to Akinkunmi and Lamikanra, (2012) the prevalence of MRSA in Pakistan, is varying from 42% to 51%. In another study, β-lactam antibiotic resistant organisms are carried by 95% of adult population in India and Pakistan (Reardon, 2014). Studies show that MRSA causes around 11,000 - 18,000 deaths, and 80,000 invasive infections yearly in the US with restricted treatment choices (Morgenstern *et al*., 2016).

Multi drug resistance (MDR) is defined as resistant to at least one agent in three or more antimicrobial classes, is the most pronounced feature of MRSA isolates as they showing sensitivity only to the glycopeptides antibiotics including vancomycin (Diaz *et al*., 2018). Conversely, vancomycin resistance has been reported in Pakistan as well (Rajaduraipandi *et al*., 2006).

In Pakistan, which spends only a small portion of its economy on healthcare, epidemics could drive the country to a catastrophe (Hussain, 2015). For the control and prevention of such infections, knowledge about the antibiotic resistance levels of clinically isolated strains is crucial. Our present study was based on the evaluation of

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the sensitivity value of clinically isolated *Staphylococcus aureus* against different antimicrobial agents currently prescribed by physicians for its control.

MATERIALS AND METHODS

Collection of sample

Clinical isolates of *Staphylococcus* bacteria were collected from a diagnostic center in Karachi. Two hundred sixty five isolates were collected from variety of sources in such a way that no isolate was repeated. All experimental work was performed in the Department of Biotechnology, University of Karachi.

Microbial media

Nutrient Agar, Nutrient Broth, Mannitol Salt Agar, Muller Hinton Agar were used at different steps. All media used in this study were purchased from Oxoid Limited.

Identification and characterization of isolated strains

Isolated strains were initially identified by Gram-staining, followed by catalase and coagulase testing as well as Mannitol fermentation testing (Winn, 2006).

Determination of antimicrobial susceptibility by disc diffusion test

Antibiotic susceptibility discs were purchased from Oxoid Limited UK, have disk content in µg. Antimicrobial susceptibilities was determined by Kirby Bauer method (Bauer *et al*., 1966) as recommended by CLSI (2016), to test all the isolates against the panel of thirteen different antibiotics, which include Oxacillin (Ox1), Ampicillin (Amp10), Vancomycin (VA30), Gentamycin (GN10), Kanamycin A (K30), Streptomycin (S10), Erythromycin (E15), Tetracycline (TE30), Ciprofloxacin (Cip5), Clindamycin (DA2), Fosfomycin (Fos50), Cefoxitin (Fox30), and Fusidic acid (FD10). For approximate number of bacteria in liquid suspension, McFarland index 0.5 turbidity standard was used.

The identified *Staphylococcus aureus* were then tested to differentiate between MSSA and MRSA. Cefoxitin Disk diffusion test performed to classify the isolates as MRSA or MSSA, since cefoxitin resistance strains have inhibition zone ≤21mm, on Mueller Hinton agar Oxoid, UK at 33-35°C for 18-24 hours (CLSI Guidelines, 2006).

RESULTS

Identification of Isolates

Percentage of isolates from specific sample sources is representing in fig. 1. There was total 265 isolates which were tested by Gram staining and conventional biochemical testing. Out of them, two hundred fifty five (255) cultures were identified as Gram positive-catalase positive Staphylococcus, Out of 255 cultures identified as staphylococci, two hundred fifty were identified as *Staphylococcus aureus* on the basis of catalase production, coagulase production and mannitol fermentation while remaining five were identified as catalase positive, coagulase negative and Mannitol non-fermenter. Table 1 categorizes the main groups for isolated cultures.

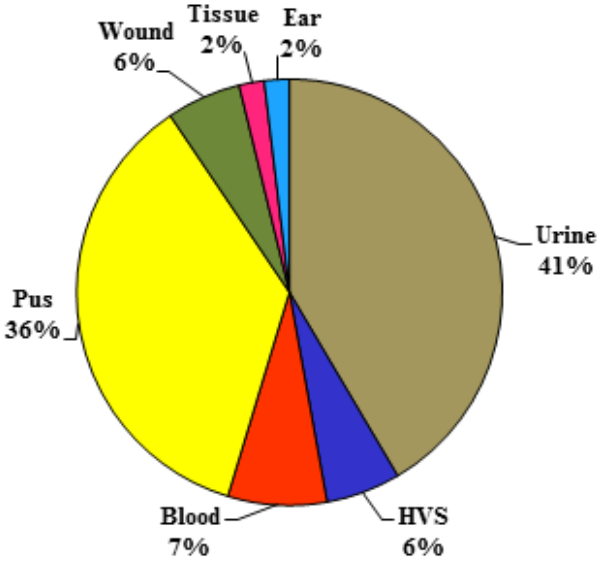

Fig. 1: Different Sources of specimen for the isolation of *Staphylococcus aureus* (n=265)

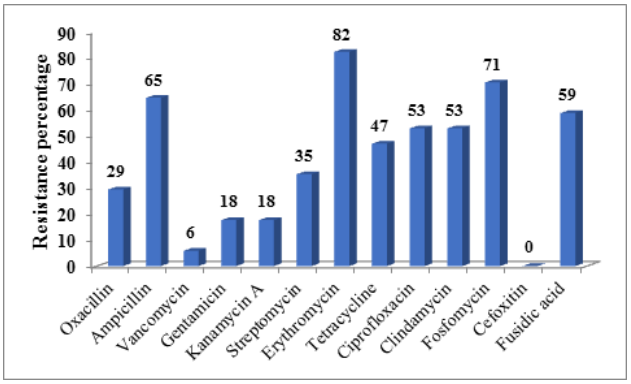

Fig. 2: Antibiotic Resistance Profile of MSSA

Antibiotic susceptibility

Disc diffusion tests showed that most isolates (88%) were resistant to ampicillin and erythromycin. Oxacillin and ciprofloxacin also showed high resistance rates i.e. 70% and 66% respectively. Most of the isolates were susceptible to vancomycin, with which only 12% resistance was observed. Cefoxitin resistance test identified 165 isolates (66%) as MRSA and 85 isolates (34%) as MSSA, out of 250 identified *Staphylococcus aureus* isolates. Fig. 2 shows the Antimicrobial resistance profile of MSSA and fig. 3 of MRSA.

The comparative study of resistance between MSSA and MRSA showed that MSSA was susceptible to most antibiotics to a greater extent than MRSA as depicted in

fig. 5. But for some antibiotics, like erythromycin, fosfomycin and fusidic acid, MSSA has also developed considerable resistance.

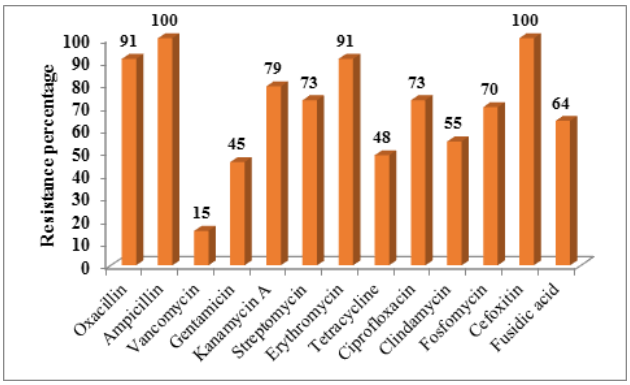

Fig. 3: Antibiotic Resistance Profile of MRSA

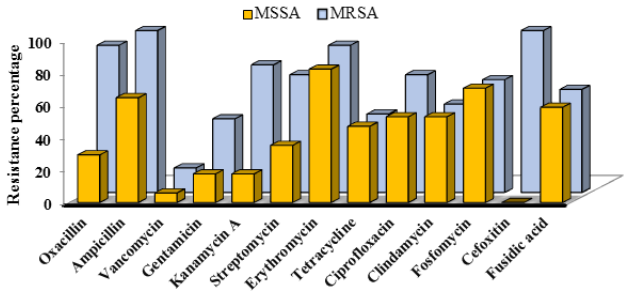

Fig. 5: Comparative illustration of Antibiotic resistance between MSSA and MRSA

DISCUSSION

Antibiotic resistance is escalating on a far greater rate than the introduction of new medicines into clinical practice, leading the world to a global health burden. Due to the widespread production and reckless use of antibiotics, a large number of microbes have developed resistance against these antibiotics, rendering these drugs useless. *Staphylococcus aureus* is a troublesome human pathogen that can cause a diverse range of infections from ordinary wound infections to life-threatening diseases like osteomyelitis, bacteraemia and heart valve infections (Diekema *et al*., 2019; Tong *et al*., 2015).

The current study has reported MRSA prevalence rate of 66% in clinical isolates of *Staphylococcus aureus*, considerably higher than the previous report that showed 52% prevalence rate of MRSA in 2017 from Karachi (Siddiqui *et al*., 2017) and form Faisalabad (Chaudary and Qureshi, 2017). Perwaiz *et al*. (2007) has reported prevalence of MRSA 50% from Lahore and 48.1% MRSA were reported from Peshawar (Vandenbroucke-Grauls, 1994). However, similar prevalence of MRSA i.e. 65%was reported in *S. aureus* isolated from ICUs in Europe (Ullah *et al*., 2016).

The antibiotic resistance pattern reveals that among the MRSA isolates, 15.1% showed resistance against vancomycin (15.1%), intermediate level of resistance was showed in cases of clindamycin (54.5%), gentamicin (45.5%) and tetracycline (48%) and the high level of resistance is encountered in cases of ciprofloxacin (72.7%) and erythromycin (91%). While in case of MSSA least level of resistance was found against vancomycin (5.8%) and gentamicin (17.6%), intermediate level of resistance was observed in case of tetracycline (47.0%), clindamycin (52.9%) and ciprofloxacin (52.9%) and high level of resistance was found in case of erythromycin (82.5%). The percentages of most of the antibiotic resistance are much higher as identified by Dibah *et al*., (2014). This demonstrates an overall elevation in the resistance pattern in both MRSA as well as MSSA, which is quite alarming and worrisome.

Resistance against kanamycin was estimated as 77% in MRSA, while 25 % in the case of MSSA. This level of resistance is higher than the results described by Ahaduzzaman *et al*., (2014). This contradiction may also suggest increasing pattern of resistance to kanamycin.

Resistance against cefoxitin in case of MSSA was 0% and all MRSA were resistant to cefoxitin while fusidic acid resistance in case of MSSA was 59% and in case of MRSA it was found to be 64%, this result is contradictory to study conducted in Peshawar, Pakistan (Ullah *et al*., 2016) and suggests an increased level of resistance to fusidic acid in MRSA strains.

In case of streptomycin, resistance percentage for MSSA and MRSA was 35% and 73% respectively which is totally different from the study of Roy *et al*. (2015), who found 0% streptomycin resistance in community acquired isolates of *Staphylococcus aureus* and 10% resistance in hospital surgery unit isolates of *S. aureus.*

In present study vancomycin resistance was encountered 6% in case of MSSA, while in case of MRSA, it came out to be 15%. This outcome is in contradiction with the findings of Hizbullah *et al*. (2015); Shah *et al*. (2016); Ullah *et al*. (2016); Hafeez *et al*. (2004) and Bukhari, (2004). All of these studies had reported 0% resistance against vancomycin. This may be an indication towards emergence of *S. aureus* with vancomycin resistance in Pakistan. This emergence of glycopeptides resistance is of great concern to our community.

According to this study, the overall antibiotic resistance trend is increasing and the time span of resistance development is alarming. All of the antibiotics tested in this study, vancomycin is the only antibiotic that can be used to treat MRSA infections at present, since it has exhibited least resistance than any of the antibiotics tested in this study. However MRSA as well as MSSA is

Table 1: Groups of Staphylococcus identified in collected samples from Laboratory

S. no.	Microorganism group	Identified number	Percentage
1	Coagulase positive *Staphylococcus aureus*	250	94.4%
2	Coagulase negative staphylococci	05	1.9%
3	Other than staphylococci	10	3.8%

evolving to develop resistance against vancomycin, as the increasing trend of vancomycin resistance suggests. Ampicillin, Oxacillin and Cefoxitin are proven to be the least successful antibiotics against the hospital isolates in this study. The present situation of antibiotic resistance must be taken into account and the rampant use of antibiotics should be prevented, otherwise it would become an uncontrollable problem to tackle these super bugs in near future.

CONCLUSION

The current proportion of antibiotic resistance in *Staphylococcus aureus* has been increasing in MRSA as well as in MSSA. Meanwhile, the number of available antibiotics for treatment of MRSA infections are limited, needs new arsenal of antibiotics to control this devastating superbug.

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