



Prevalence and antimicrobial susceptibility profile of MRSA and VRSA strains isolated from out - door patients of Swabi region, Pakistan

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Abstract

Staphylococcus aureus is one of the nosocomial pathogen, causes life threatening infection include urinary tract infection (UTI), blood stream infection and soft tissue infection. The purpose of this study was to detect Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Staphylococcus aureus* (VRSA) in outdoor patients based on antibiotic susceptibility profiling. Total of hundred different specimens i.e. pus, urine, blood and sputum were collected and analyzed in Microbiological laboratory for presence of *Staphylococcus aureus*. All specimens were inoculated on different media like MacConkey and MSA and incubated on 37°C. Disc diffusion method was used to check antimicrobial susceptible profile. Out of 100 samples *Staphylococcus aureus* was detected in 17 samples. Among these 17 positive samples four were MSSA, nine were both MRSA and VRSA and 4 isolates were VRSA. Among all isolates 64.71% were resistant of gentamycine, 23.53% to cefoxitin, 23.53% to vancomycin, 58.82% to erythromycin, 100% to pencilline and 52.94% to levofloxacin. Whereas all isolated strains were sensitive to linezolid only. It is concluded that MRSA and VRSA are prevalent in outdoor patients and linezolid is only antibiotic which is susceptible to all isolates.

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Introduction

Staphylococcus aureus is main pathogen of human, and spread widely in nature. *S. aureus* is coagulase positive, gram positive, catalase positive, facultative anaerobic and microscopically characterized as a single, pairs or clusters cocci, non-motile and non – sporulating bacteria (Leticia JG *et al.*, 2003). It has been recognized from more than hundred years that *S. aureus* cause human infections. The infection collection from negligible skin infection up to major bone diseases i.e endocarditis and septicemia (Howden B.P *et al.*, 2010).

Before antibiotics discovery, the rate of morbidity was higher than 75% because of *S. aureus* infections (Van S.J *et al.*, 2012). But with the discovery and the successful use of penicillin, in treating *S. aureus* infections, new strain began to appear against this drug (Atkinson B.A *et al.*, 1984). And today about 90% strains of *S. aureus* are resistant to penicillin (Chambers H.F 2001). Since last few decades, resistance to methicillin, oxacillin and all other β -Lactam drugs in *S. aureus* is increasing day by day (National Nosocomial Infection Surveillance 2003). Now MRSA is the important cause of hospital and community acquired infections globally (Deurenberg R.H and Stobberingh E.E., 2008). The first choice treatment of MRSA infection was vancomycin. But now it is reported from different part of the world that now *Staphylococcus aureus* is been resistant to vancomycin. In 1996 for first time it was reported from Japan that susceptibility to antibiotic vancomycin is reduced (Thati V *et al.*, 2011; Pierard D *et al.*, 2004). The present study was conducted to evaluate the prevalence and antibiotic susceptibility pattern of MRSA and VRSA isolated from different clinical specimens of district Swabi Pakistan.

Materials and methods

Specimen Collection

The current study was done in Microbiology Laboratory of University of Swabi from Nov 2016 to May 2017. A total of 100 various clinical specimens were collected from OPD patient. In clinical specimens' blood, urine, pus and sputum were included.

Culture preparation and identification

All the specimens were inoculated on nutrient agar media and on MSA (oxide-UK) to obtain the growth and plates were incubated at 37°C for 24hr. In case of growth, for the identification of colony, gram staining and other biochemical tests were done i.e. Catalase, coagulase and Dnase (oxide-UK). Among all isolated strains only *S.aureus* isolates were included in the study.

Antimicrobial susceptibility testing

According to the CLSI guidelines antimicrobial susceptibility test was done by using MHA (Oxide-UK). The antibiotics used against *S. aureus* isolates were Penicillin (10 μ g), Gentamicin 10 μ g), Erythromycin (10 μ g), Doxycycline (30 μ g), Vancomycin (30 μ g), cefoxitin (30 μ g), Linzolid (15 μ g), Levofloxacin (5 μ g) and Clindamycin (2 μ g).

Results

Prevalence of staphylococcus aureus in outdoor patients

Hundred different specimens i.e. urine, blood and pus were collected from OPD patients. Out of total only from 17(17%) specimen *S.aureus* were isolated. Whereas *E.coli*, *Pseudomonas aeroginose* and *proteus vulgaricus* were present in 49 (49%), 10 (10%), 6 (6%) and 5 (5%) respectively. Among total specimens 13 (13%) showed no growth (Table 1).

Table 1. Rate of Prevalence of various bacteria in Specimens.

Bacteria name	Total number (n=100)	Percentage n(%)
<i>E.coli</i>	49	49%
<i>Staphylococcus aureus</i>	17	17%
<i>Klebsiella pneumoniae</i>	10	10%
<i>Pseudomonas aeroginosa</i>	6	6%
<i>Proteus vulgaricus</i>	5	5%
No growth	13	13%

Characterization and distribution of MRSA and VRSA

Among total 17 isolated *S. aureus* 13(76.4%) were detected as MRSA and out of these 13 isolates 4

(23.5%) were both MRSA/VRSA and 9 (52.9) were MRSA. while only 4(23.5%) isolates were Methicillin sensitive *Staphylococcus aureus* (MSSA) (Table 2).

Table 2. Distribution and characterization of different *S.aureus* strains.

Total strain and percentage	17 (100%)
Number and percentage of MRSA and VRSA	4 (23.5%)
Number and percentage of MRSA	9 (52.9%)
Number and percentage of MSSA	4 (23.5%)

Gender Based Distribution of *Staphylococcus aureus*

Based on gender out of total 4 isolates MSSA distribution was only 1(25%) in male and the 3(75%) in female. While in total of 13 MRSA and MRSA/

VRSA strains n= 11(84.6%) were isolated from males and 2(15.38%) were collected from females specimens (Table 3).

Table 3. Gender based distribution of *Staphylococcus aureus*.

<i>Staphylococcus aureus</i> Strains	Gender	
	Male	Female
MSSA (n=4)	1(25%)	3(75%)
MRSA+MRSA/VRSA (n=13)	11(84.6%)	2(15.38%)

Table 3 Continued. Number and percentages of MSSA, MRSA and MRSA/ VRSA collected from different specimens (Total n |=17).

Specimen	Number and % of MSSA n(%)	Number and % of MRSA	Number and % of VRSA/MRSA
Urine	2(11.76%)	—	1(5.88%)
Blood	—	—	—
Pus	2(11.76%)	9(52.94%)	3(17.64%)
Sputum	—	—	—

Percentage of MSSA, MRSA and MRSA/ VRSA in different specimens

In total 17 strain of *S. aureus* MSSA isolated from urine was 2(11.7%) and from pus is 2(11.7%). While MRSA and VRSA from urine was 1(5.88%) and from pus was 13 (76.4%). Whereas MRSA/ VRSA both i.e. 1(5.88%) from urine and 3(17.64%) from pus. While from blood and sputum no *S. aureus* strain was isolated (Table 4).

Antibiotic profile of mrsa, vrsa and mssa

For antibiotic susceptibility testing seven different antibiotics were used i.e. linezolid, Gentamicin, cefoxitin, Vancomycin, erythromycin, penicillin, and

Levofloxacin. All 17 isolates of *S.aureus* were distributed in MSSA, MRSA and MRSA/VRSA with 4 strains, 9 strains and 4 strains respectively. Among total 4 MSSA strains 4(100%) were sensitive to three antibiotics Linezolid, Cefoxitin and Vancomycin, 3(75%) to Levofloxacin, 2(50%) to erythromycin, 1(25%) to gentamycin and 4(100%) to resistant to penicillin, While out of total 9 MRSA strains all were (100%) resistant to Cefoxitin and Penicillin, 6(66.6%) to Levofloxacin, 5(55.5%) to Gentamycin, 4(44.4%) Erythro-mycin and 100% sensitive to Linezolid and vancomycin. Similarly all 4 MRSA/VRSA strains were 100% resistant to Cefoxitin, vancomycin, Erythromycin, Penicillin, 3(75%) to Genamycin and

Levofloxacin and 4(100%) sensitive to linezolid (Table 5)

Discussion

In this study prevalence and antibiotic susceptibility of MRSA and VRSA from outdoor patient of Swabi Hospital was evaluated. The rate of MRSA from

different clinical sample is 76.4% (n=13) and out of these 76.4% only 29.4% were VRSA. Clinical samples contain pus, blood, sputum and urine. According to Heather M in Europe there is 65% MRSA infection (Heather M. 2009). In India the prevalence of MRSA infection is very high which is up to 71% in 2008 from pus or wounds (Tambekar DH., *et al* 2008).

Table 4. Antibiotic susceptibility profile of *S.aureus* strain.

Antibiotics	MSSA			MRSA			MRSA/VRSA		
	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Linezolid	—	—	4(100%)	—	—	9(100%)	—	—	4(100%)
Genta-mycin	3(75%)	1(25%)	—	5(55.5%)	1(11.1)	3(33.3%)	3(75%)	—	1(25%)
Cefoxitin	—	—	4(100%)	9(100%)	—	—	4(100%)	—	—
Vanco-mycin	—	—	4(100%)	—	—	9(100%)	4(100%)	—	—
Erythro-mycin	2(50%)	—	2(50%)	4(44.4%)	2(22.2%)	3(33.3%)	4(100%)	—	—
Pencillin	4(100%)	—	—	9(100%)	—	—	4(100%)	—	—
Levo floxacin	—	1(25%)	3(75%)	6(66.6%)	—	3(33.3%)	3(75%)	—	1(25%)

R= Resistant, I= Intermediate, S= Sensitive.

In Pakistan the prevalence of MRSA is uneven. On average the prevalence of MRSA infection is 42% (Romeeza T *et al* 2009). In different zones and areas different studies was conducted at different time, which shows inconsistent rates. In 1999 a study was conducted Sargodha and result showed that there were 23% MRSA in different clinical samples (Siddiqi GM *et al* 2009). From clinical samples in Lahore from Mayo hospital 38.5% MRSA were isolated in 2001. Another study was conducted in Karachi in 2005 the result was 43% out of 190 strain of *S. aureus* (Perwaiz S *et al* 2007). Samples were isolated from Pus in Lahore in 2008 and the rate of MRSA is 40%. Our study shows that the rate of MRSA from Pus is 76.4%. Vancomycin Resistance has led to alarming situation worldwide as Vancomycin is fit planned as the last successful drug to treat the Staphylococcus infections. Up to now, it has been reported in a lot of countries including Pakistan about intermediate Vancomycin resistance. Our study indicates that Vancomycin is 23.5% of total *S. aureus* and 4% of total samples in OPD patients of distract Swabi. According to one report, the prevalence of ViSA is 13%. (Sonavane A *et al* 2007) and same study was conducted in 2008 and

result recorded as 7.5% (Mehdinejad M, *et al* 2008). Different clinical sample were isolated from August 2010 to July 2011 in Dhaka Bangladesh.

They detects no VRSA but report a shocking result in which 6.56 % *S.aureus* show sensitivity to Vancomycin and 93.44 % were intermediate (Shahriar M *et al* 2012).

According to 2006 the VRSA occurrence is very different from one hospital to another. The occurrence of VRSA in Jinnah Hospital was reported as 0%, in Services Hospital the occurrence rate is 1%, the rate in Mayo hospital is 2% and same is the result of Ganga Ram Hospital (Bataineh AB 2006). But our study reported that the rate of VRSA is 5.88%.

The total MSSA strain sensitive to Gentamicin. Trivedi MB, *et al.*, reports that 85.6% MSSA strain were sensitive to Gentamicin. Shrikanth, *et al.* reported in 2013 that it's 91.8% (Mir BA, Shrikanth. 2013). In our result Erythromycin is 38.4% sensitive to MRSA. But Goyal A, *et al.*, reported in 2013 that the result is 67.3% (Goyal A *et al.*, 2013).

FOX were resistance to all MRSA strain. Vancomycin was resistance to 5 isolated which were found as VRSA. Isolated were also found resistance to too many antibiotic like Penicillin and somewhat to Levofloxacin. In the current study all the MRSA and VRSA isolates were sensitive to linezolid. Only linezolid were found that it is effective against all VRSA isolates. Similar results were also reported by Kaleem *et al* in 2010. In 2001, Gemmell CG also reported that linezolid is 100% susceptible to *S.aureus* infection (Gemmell CG., 2003).

Conclusion

In this study rate of prevalence of *S. aureus* was recorded in outdoor patients, it was over all 17%. Furthermore MSSA and MRSA/VRSA strains were isolated from female and male patients specimens respectively. All strains showed high resistance to more than three drugs while linezolid was found only antibiotic highly effective against all strains isolated.

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