Antibiotic Sensitivity to Methicillin and Methicillin Plus Macrolide-Lincosamide-Streptogramins Resistant Staphylococcus Aureus

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Abstract

This study was conducted from May 2018 to Oct 2019 to determine the culture and sensitivity pattern of Methicillin resistant Staphylococcus aureus (MRSA) and MRSA with Macrolide Lincosamide and Streptogramin-B (MLS-B) antibiotics in a tertiary care hospital of Nowshera.

A total of 235 isolates were studies and 86 samples showed MRSA. The prevalence rate of MRSA was 89/235 (37.87%). Out of 86 isolates with MRSA, 19 (22.09%) were also resistant to MLS-B antibiotics. Regarding gender specification, 52 (60.5%) were females and 34 (39.5%) were males. The mean age with standard deviation of patients was 29 + 6.8 years. The sensitivity pattern to MRSA was; Vancomycin 100%, Linezolid 100%, Rifampicin 86.04%, Clindamycin, 73.25%, Fusidic acid 60.46%, Erythromycin 44.18%., Doxycyclin 32.55%, levofloxacin 16.27%, Gentamycin 13.95% and Ciprofloxacin 11. 62%. Sensitivity of Vancomycin & Linezolid was 100% in MRSA and MLS-B resistant isolates, and should be kept reserved for MRSA cases to avoid misuse of antibiotics and to reduce resistance.

Keyword: Antimicrobial resistance (AMR), MRSA, MLS-B, Misuse of antibiotics.

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Introduction

Antimicrobial resistance (AMR) is a serious global health concern, specially reported with Gram-positive bacteria, the most notorious bug, MRSA, which is an emerging threat[1].

Infections associated with Methicillin-resistant Staphylococcus aureus (MRSA) is a major global healthcare issue and challenging the clinicians to treat such type of infections. S. Aureus bacteremia, exhibits a very high rate of morbidity and mortality worldwide and can lead to life threatening conditions like infective endocarditis or sepsis by metastasis[2]. Resistance to methicillin is mediated by mec-A gene, which encodes the polypeptide PBP2a protein[3].

The timely advised antibacterial therapy has better outcomes in treatment of methicillin resistant Staph Aureus infections. Bacteremia caused by methicillins sensitive Staph Aureus i.e. MSSA, therapy of choice is beta-lactam antibiotics with acceptable results but for infections caused by MRSA srains, the antibiotics of choice are vancomycin or daptomycin as per recommendations of the Infectious Diseases Society of America guidelines and many other meta analysis[4-5]. Clindamycin is an important drug duly approved by FDA and is used to treat MRSA infections. Unlike other antibiotics such as doxycyclin, trimethoprim, sulfamethoxazole, rifampin, and linezoid, its use has been less restrained by safety considerations and precautions[6].

Vancomycin and linezolid are used are gold standard drug for MRSA associated bactermia. Concern associated with Vancomycin is that its bactericidal activity is relatively slow and it poorly penetrates some tissues[7]

Present study was conducted to determine the antimicrobial sensitivity of MRSA and MRSA with MLS-B isolates in resistant isolates in a hospital-based study.

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Patients and Methods

This Cross-sectional study was performed in the Pathology department of Qazi Hussain Ahmed Medical Complex Nowshera from 1st May 2018 to 30th Oct 2019. A total of 235 isolates were studies and 86 samples were reported with MRSA (36.59%). Out of 86 cases with MRSA, 19 (22.09%) were also resistant to MLS-B antibiotics. Sample size of 235 was calculated on the following assumption that the anticipated proportion of the MRSA is at 39%. The absolute precision was 6%, confidence level 95% and a drop Outs. (lost to follow up) up to 10%[8].

The inclusion criteria were all cases irrespective of age and gender received in the laboratory. Exclusion criteria were samples received in the laboratory 24hour after collection, patient already on the antibiotic therapy and improperly collected sputum and pus samples.

The samples were received in the pathology section from the respective unit under observance of strict aseptic technique after education of patients on pus and sputum sample collection. Media were prepared as per CLSI (Clinical and laboratory standard institutes).

All samples were inoculated on selective medium MSA (Mannitol Salt Agar). Then the specimens were Incubated under ambient air 35 +2 C for 18-20 hours. In case growth is obtained on MSA then further inoculated on Mueller Hinton agar for sensitivity to antibiotics as per CLSI recommendations. The antibiotic desks used were; VA-Vancomycin, LZD-Linezolid, RD-Rifampicin, D-Clindamycin, E-Erythromycin, Fd-Fusidic acid, Dox-Doxycyclin, Lev- Levofloxacin, CN-Gentamycin, Cip-Ciprofloxacin, SXZ (Cotrimaxazole) and Fox-Cefoxetin.

The accepted zones of sensitivity taken in consideration as per CLSI (Clinical Laboratory Standard Institute) Guideline 2015[9] for different antibiotic desks were: Linezolid >21mm, Vancomycin>20mm (Now MIC Test is recommended in fresh CLSI), Rifampicin>20mm Clindamycine>21mm, tetracycline>19mm, ciprofloxacin>21mm, levofloxacin>19mm, Gentamycin >15mm and CXZ (Trimethoprim Sulfamethoxazole)>16mm.

For recognition of MLS-MRSA, or MRSA with further resistance to MLS-B antibiotics, the desk of Erythromycin and Clindamycin were placed at a distance of 20mm center to center. Phenotypically MSL resistance was confirmed as Inhibition of zone of clindamycin towards erythromycin as a straight line, resembling the alphabet "D" and was considered to be positive for D-Test phenomenon (Figure 1). Any haziness in the zone of inhibition of clindamycin is also phenotypically representative of resistance. Finally, the data obtained from the culture and sensitivity was entered in a SPSS version 25 for descriptive analysis of different parameters.

Results and Discussion

Out of 235 patients, 86 MRSA Positive isolates (36.59%) were selected for antibiotic susceptibility. Out of total, 52 (60.5%) were females and 34 (39.5%) were males, with male to female ratio of 1.4:1 (figure 1). Continuously Emerging resistance to available antibiotics by MRSA is a global threat and challenge to the clinicians in both clinical facilities and community settings[7,8]. We observed the prevalence of MRSA in teaching hospital set up as (36.59%) among the total Staph aureus isolates. MRSA is reported worldwide nearly from all regions of the world with variable frequencies. The frequency of MRSA in Pakistan and other neighboring countries like India has been shown to be high if compared with developed world like USA and Europe. Many factors can contribute to this difference[10]. A study reported from Africa showed the prevalence of methicillin resistance in S. aureus from 42 to 51%[11] that quit matching our findings. Another local study from Peshawar KP Province, reported that the frequency of MRSA to be 39.8 and 34 % for female and male respectively[12].

The mean age of patients was 29 + 6 years. The minimum age of the patients was 20 years with maximum of 45 years with age range of 25 years (Table 1). Our findings do match with the findings of other national and international studies conducted on the same theme[10-12].

The sensitivity to Vancomycine & Linezolid was 100% in MRSA isolates. (Table 2). Sensitivity to the next antibiotic showed higher sensitivity was: Rifampicin 86%, Clindamycin, 73.25%, Erythromycin

44.18% and Doxycyclin was 32.55%. Sensitivity to quinolones and flouroquinolones was not remarkable. Similarly, no sensitivity was recorded for cotrimaxazole in MRSA in present study. A local study reported from the major cities of the Pakistan that 100% of the positive isolates of MRSA were sensitive to Vancomycin with resistant to all third generatio antibiotics. Sensitivity graph to other commonly prescribed antibiotics varied from 20-52%[12]. Ullah A et al[8] also reported that that all MRSA isolates were 100% sensitive to linezolid and vancomycin followed by rifampicin (81.2 %), chloramphenicol (77.2 %), clindamycin (75.2 %), minocyclin (67.3 %).

The sensitivity to clindamycine was 73% due to expression of erm gene encoding Methylase by erythromycin confirmed phenotypically by D-test Phenomenon in-vitro.

Table 1. Age Statistics

Number of patients	86
Mean	29
Median	25
Std. Deviation	6.8
Range	25.00
Minimum	20.00
Maximum	45.00

Table 2. Sensitivity pattern to MRSA positive isolates.

Antibiotics	Sensitivity to MRSA Positive Cultures	Total Sensitivity	%
Vancomycine	86	86	100
Lanezolid	86	86	100
Rifampicin*	74	86	86.04
Clindamycine	63	86	73.55
Fusidic acid*	52	86	60.46
Erythromycine	38	86	44.18
Doxycycline	28	86	32.55
Levofloxacine	14	86	16.27
Gentamycin*	12	86	13.95
Ciprofloxacin	10	86	11.62
Cefoxetin(b-lactam antibiotics)	0	86	0.00
Cotriamaxazole	0	86	0.00

*But is not indicated as mono-therapy and must be given in combination with another antibiotic with sensitivity to the specific isolate and with different mechanism of action[7].
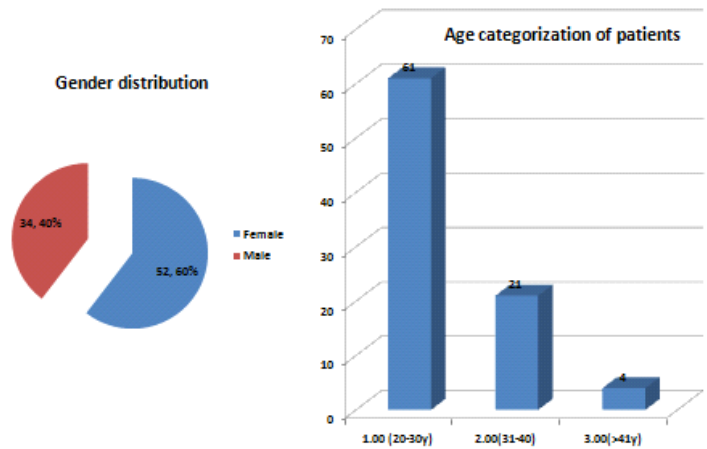

Figure 1: the age and gender categorization of patients

Furthermore, out of 86 isolates with MRSA, 19 (22.09%) were also resistant to MLS-B antibiotics. Clindamycin and streptogramin are golden drugs used for treating infections induced By Methicillin Resistant S. Aureus (MRSA). Therefore, resistance to this precious antibiotic in the shape of D-test is annoying and challenging[8]. There is need for legislations and also administratively to control the irrational use of prestigious antibiotics like vancomycin and linezolid. Developed countries have developed strategies for the use of vancomycin in clinical practice, an example is USA where they have designed a computerized structured system in hospital to bound theclinicians starting vancomycin for treating resistant infections, where the clinicians were supposed to follow a protocol with clear mention of proper indication of vancomycin and updating the treatment record in the a computerized interconnected system to be strictly observed by the decision makers under Management information system to avoid its misuse[13].

Vancomycin being the treatment of choice for MRSA. But due to irrational and unlawful use of this golden molecule has led to emergence of new S. aureus strains as reported in the literature called vancomycin-intermediate S. aureus (VISA) and heterogeneous-VISA (hVISA). MRSA can also show

minimal resistance by mechanism of tolerance to vancomycin, that can be confirmed by minimum inhibitory concentration (MIC) ratio of ≥32. The infections caused by VISA, hVISA and vancomycin-tolerant MRSA (VT-MRSA) are very difficult to treats especially in conditions when they cause endocarditic particularly in immune-compromised patients like HIV etc[10-12].

A comprehensive strategy using advocacy, communication social mobilization and CME events can help in understanding healthcare provider in proper selection of antibiotics for treatment of MRSA infections.

There is need for multidisciplinary approached at national, international levels to control the unlawful and similarly irrational use of antibiotics. evidence based use of drug should be encouraged to safeguard the future clinical challenges.

Conclusion

It is concluded the prevalence of MRSA is 89/235 (37.87%). While 19/88 (22.09%) of the MRSA isolates were also resistant to MLS-B. Sensitivity of Vancomycin & Lanezolid was 100% in MRSA and MLS-B resistant isolates, and should be kept reserved for MRSA cases to avoid misuse of antibiotics. There is need for legislative changes to ensure the use of appropriate antibiotics after culture and sensitivity.

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