DETERMINATION OF VANCOMYCIN AND LINEZOLID RESISTANCE IN STAPHYLOCOCCUS AUREUS ISOLATED FROM KATIHAR DISTRICT OF BIHAR, INDIA

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ABSTRACT

BACKGROUND

Staphylococcus aureus is a major human pathogen world-wide. Staphylococcus aureus strains with decreased susceptibility to vancomycin and linezolid among methicillin-resistant Staphylococcus aureus (MRSA) have been reported from different parts of world. Linezolid is the only antibiotic as an oral formulation for resistant staphylococcal infection including MRSA and VRSA. Vancomycin and linezolid resistance is a serious issue because these antibiotics are the only reliable option for the treatment of MRSA infections.

The aim of the study was to assess vancomycin and linezolid-resistant Staphylococcus aureus in Katihar District of Bihar.

MATERIALS AND METHODS

All Staphylococcus aureus were identified as per standard protocol and antibiotic susceptibility tests were put up using a panel of antibiotics. Minimum inhibitory concentrations (MICs) of vancomycin (1 µg/mL- 64 µg/mL) and linezolid (1 µg/mL-32 µg/mL) were then determined by agar dilution method.

Statistical Analysis- http://www.physics.csbsju.edu/cgi-bin/stats/contingency[1]

RESULTS

Out of the 108 Staphylococcus aureus isolates, 32 (29.6%) were found to be methicillin resistant by the cefoxitin disc diffusion method, 9 (8.33%) were found to be vancomycin-resistant Staphylococcus aureus (VRSA) and 4 (3.70%) linezolid-resistant Staphylococcus aureus (LRSA) by disc diffusion method. Out of the 9 suspected strains of VRSA, 4 (3.70%) were confirmed as VRSA; out of 4 suspected strains of LRSA, 2 (1.85%) were confirmed as LRSA. 11 (10.19%) were found to be vancomycin-intermediate Staphylococcus aureus (VISA) by MICs method.

CONCLUSION

VRSA and LRSA in this region was found to be relatively low (3.7% and 1.9% respectively of all Staphylococcus aureus isolates), which is probably because the medical college is situated in rural area where the organisms are not exposed to as much antibiotic pressure as in the urban areas. All VRSA and LRSA were isolated from indoor patients. None of the VRSA and LRSA were community associated, which may be because these strains develop in hospital settings under high antibiotic pressure. Tests need to be performed in the laboratories to detect VRSA and LRSA as this will prevent random use of reserve drugs like vancomycin and linezolid except in those cases where all other drugs are resistant.

KEYWORDS

Staphylococcus Aureus, VRSA, LRSA.

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BACKGROUND

Staphylococcus aureus is a major human pathogen world-wide, causing a variety of infections ranging from localised skin infections to life-threatening infections. Over the past few decades, methicillin-resistant Staphylococcus aureus (MRSA) strains have become endemic in hospitals worldwide. In addition to β-lactams, most of these strains are also resistant to glycopeptides (vancomycin) and oxazolidinone (linezolid) antibiotics.[2] Vancomycin has been regarded as the first-line drug for treatment of MRSA. Unfortunately, there has been an increase in the use of this antibiotic for other infections, such as pseudo-membranous colitis caused by Clostridium difficile and coagulase-negative staphylococcal infections in hospitalised patients. When this drug was introduced in 1858, it was perceived that there would be no resistance to this antibiotic. However, in 1997 the first strain of Staphylococcus aureus with reduced susceptibility to vancomycin was reported from Japan. Since then, there has been an increase in the number of cases with both

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vancomycin-intermediate Staphylococcus aureus (VISA) and vancomycin-resistant Staphylococcus aureus (VRSA). This has led to a greater number of life threatening infections due to Staphylococcus aureus in both hospitalised and non-hospitalised patients.[3] Staphylococcus aureus strains with decreased susceptibility to vancomycin have been reported from different part of world. Subsequently, VISA isolates were reported from U.S. and other countries including Brazil, United Kingdom, Germany, India and Belgium which has confirmed that the emergence of these strains is a global issue. Vancomycin resistance in VISA isolates was acquired by the thickening of the cell wall due to the accumulation of excess amounts of peptidoglycan.[2] One VRSA strain was reported from Michigan and one from Pennsylvania contained the vanA gene, which codes for an altered target D-Ala-D-Lac instead of D-Ala-D-Ala.

As per Clinical Laboratory Standards Institute (CLSI), Staphylococci with minimal inhibitory concentrations (MICs) of vancomycin <2 µg/mL should be considered sensitive, while those for which the MICs is 4-8 µg/mL should be considered intermediate sensitive and those with MIC >16 µg/mL should be reported as resistant.[4]

Linezolid is a synthetic inhibitor of protein synthesis that is active against many Gram-positive pathogens including MRSA and VRSA, vancomycin-resistant enterococci (VRE), Streptococcus species and penicillin-resistant pneumococci.[5]

Linezolid was approved by the US Food and Drug Administration in 2000 for the treatment of uncomplicated and complicated skin and soft-tissue infections, including diabetic foot infections without concomitant osteomyelitis, community-acquired and nosocomial pneumonia and vancomycin-resistant Enterococcus faecium infections, including cases with concurrent bacteraemia. Activity against Nocardia spp. and Mycobacterium spp. has also been demonstrated, including cases of central nervous system infection and infective endocarditis.[6]

The mode of action of linezolid is different from that of other protein synthesis inhibitors which prevent protein synthesis at the chain elongation step. However, linezolid prevents the 50S subunit of prokaryotic ribosome to complex with the 30S initiation complex and inhibits bacterial protein synthesis at the initiation step of protein biosynthesis. The expression of virulence factors in toxin-producing Staphylococcus aureus is sensitive to the inhibition of protein synthesis by linezolid. With this novel mechanism of action by linezolid, it was thought that bacteria would never develop resistance to linezolid.[7]

Retaining the efficacy of these two antibiotics is important as vancomycin and linezolid are the only reliable option for the treatment of MRSA infections.

Taking into consideration the menace that is VRSA both in hospital and more recently in community settings and also the emergence of LRSA, this study was undertaken to determine the presence of VRSA and LRSA among Staphylococcus aureus isolates in Katihar District, Bihar.

MATERIAL AND METHODS

Patients of both sexes and all age groups were included in this study after obtaining Institutional Ethical Committee clearance and informed consent from each and every patient.

Specimens were collected as per standard protocol. All samples were subjected to microscopic examination and cultured on standard laboratory media.[8]

Staphylococcus aureus were identified as per standard protocol.[8] Antibiotic susceptibility tests were put up by modified Kirby-Bauer's disc diffusion method using a panel of antibiotics obtained from HiMedia (Mumbai).[4]

Detection of MIC by Agar Dilution Method

Minimum inhibitory concentrations (MICs) of vancomycin (1 µg/mL- 64 µg/mL) and linezolid (1 µg/mL- 32 µg/mL) were then determined by agar dilution method.[4]

The gradient plates of Muller-Hinton agar were prepared with different concentrations of vancomycin and linezolid.[9] Vancomycin and linezolid powder in pure form was procured from Sigma-Aldrich, India.

The test organisms were emulsified in sterile normal saline and the turbidity was matched with 0.5 McFarland's standards. The bacterial strains were then spot inoculated on the surface of agar medium using 10 µL of bacterial culture. The plates were incubated at 35°C for 24 hours and subsequently observed for any visible growth. More than one colony or light film of growth indicated reduced susceptibility. The minimum concentration of the antibiotic, which was able to inhibit bacterial growth, was considered as MIC. Results were read and interpreted according to CLSI guidelines.[4]

For vancomycin, strains with MIC <2 µg/mL were reported as susceptible, those with MIC between 4 and 8 µg/mL were reported as intermediate and strains with MIC > 16 µg/mL were reported as resistant.

For linezolid, strains with MIC <4 µg/mL were reported as susceptible and those with MIC >8 µg/mL were reported as resistant.

Ethics

Institutional Ethics Committee clearance was obtained vide letter No. IEC/IRB No: KMC/IEC/2014-2017/017/MD (Micro)

Statistical Analysis was done using an online software.[1]

RESULTS

A total 3056 clinical samples were received in the Microbiology laboratory from January 2015 to June 2016, out of which 108 were Staphylococcus aureus. Majority of Staphylococcus aureus strains isolated during the study period were from the age group 21-30 years - 39/108 (36.1%), followed by age group 11-20 years - 23/108 (21.3%).

The total number of female patients from whom Staphylococcus aureus was isolated was 71/108 (65.7%) as compared to males 37/108 (34.3%).

Out of these 108 strains of Staphylococcus aureus, 9 (8.33%) were VRSA and 4 (3.70%) were LRSA by disc diffusion method while 11 (10.2%) were VISA, 4 (3.70%) were VRSA and 2 (1.85%) were confirmed as LRSA by agar dilution MIC method. This finding was not found to be statistically significant. (Table 1).

Out of the 108 strains of Staphylococcus aureus, 76 were MSSA and 32 were MRSA. None of the MSSA was found to be either vancomycin intermediate or vancomycin resistant. Out of the 32 strains of MRSA, 17 were vancomycin sensitive, 11 were intermediate and 4 were VRSA. (Table 2).

Linezolid resistance was seen in only two out of the 108 strains of Staphylococcus aureus. Out of the 106 strains that

had MIC 1-4 µg/mL, 76 (71.70%) were MSSA and only 30 (28.30%) were MRSA. The two strains that were linezolid resistant were found to be MRSA. (Table 3).

Amongst the VSSA strains maximum resistance were seen with amoxicillin 89/93 (95.70%) followed by gentamicin 73/93 (74.49%). VISA and VRSA strains showed maximum resistance to amoxicillin, cefotaxime, cefoxitin and cefuroxime to which 100% strains were resistant. All VISA strains were sensitive to linezolid and all VRSA strains were sensitive to imipenem. (Table 4).

Method (n = 108)	VISA (%)	VRSA (%)	LRSA (%)
Disc diffusion	NA	09 (08.33)*	04 (03.70)†
Agar dilution	11 (10.19)	04 (03.70)*	02 (01.85)†

Table 1. Correlation between Different Methods for Detection of VRSA AND LRSA

*p value- 0.153 †p value- 0.408

Strains	Concentration of vancomycin						
	1 µg/mL (%)	2 µg/mL (%)	4 µg/mL (%)	8 µg/mL (%)	16 µg/mL (%)	32 µg/mL (%)	64 µg/mL (%)
MSSA (n =76)	13 (92.86)	63 (79.75)	00 (00.00)	00 (00.00)	00 (00.00)	00 (00.00)	00 (00.00)
MRSA (n=32)	01 (07.14)	16 (20.25)	03 (100.0)	08 (100.0)	01 (100.0)	02 (100.0)	01 (100.0)
Total (n= 108)	**14**	**79**	**03**	**08**	**01**	**02**	**01**

Table 2. MIC of Vancomycin of Staphylococcus aureus Strains

Strains	Concentration of linezolid					
	1 µg/mL (%)	2 µg/mL (%)	4 µg/mL (%)	8 µg/mL (%)	16 µg/mL (%)	32 µg/mL (%)
MSSA (n =76)	06 (75.00)	21 (77.78)	49 (69.01)	00 (00.00)	00 (00.00)	00 (00.00)
MRSA (n =32)	02 (25.00)	06 (22.22)	22 (30.99)	01 (100.0)	01 (100.0)	00 (00.00)
Total	**08**	**27**	**71**	**01**	**01**	**00**

Table 3. MIC of linezolid of Staphylococcus aureus Strains

Antibiotic	VSSA (n =93)		VISA (n =11)		VRSA (n =04)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Amikacin	78 (83.87)	15 (16.13)	10 (90.91)	01 (09.09)	03 (75.00)	01 (25.00)
Amoxicillin	04 (04.30)	89 (95.70)	00 (00.00)	11 (100.0)	00 (00.00)	04 (100.0)
Cefotaxime	55 (59.14)	38 (40.86)	00 (00.00)	11 (100.0)	00 (00.00)	04 (100.0)
Cefoxitin	76 (81.72)	17 (18.28)	00 (00.00)	11 (100.0)	00 (00.00)	04 (100.0)
Cefuroxime	39 (41.94)	54 (58.06)	00 (00.00)	11 (100.0)	00 (00.00)	04 (100.0)
Clindamycin	53 (56.99)	40 (43.01)	08 (72.73)	03 (27.27)	03 (75.00)	01 (25.00)
Gentamicin	20 (21.51)	73 (78.49)	03 (27.27)	08 (72.73)	01 (25.00)	03 (75.00)
Imipenem	83 (89.25)	10 (10.75)	10 (90.91)	01 (09.09)	04 (100.0)	00 (00.00)
Linezolid	93 (100.0)	00 (00.00)	11 (100.0)	00 (00.00)	02 (50.00)	02 (50.00)
Teicoplanin	81 (87.10)	12 (12.90)	02 (18.18)	09 (81.82)	00 (00.00)	04 (100.0)
Tobramycin	39 (41.94)	54 (58.06)	08 (72.73)	03 (27.27)	02 (50.00)	02 (50.00)

Table 4. Antibiotic Susceptibility Pattern of VSSA, VISA & VRSA

DISCUSSION

Out of the 108 strains of Staphylococcus aureus, 32 (29.6%) were MRSA, 9 (8.33%) were VRSA and 4 (3.70%) were LRSA by disc diffusion method. Out of these 9 strains of VRSA, only 4 (3.70%) were confirmed as VRSA and out of 4 strains of suspected LRSA, only 2 (01.9%) were confirmed as LRSA by the MIC method. Moreover another 11 strains (10.19%) were confirmed as VISA by MICs method.

In this study, overall male to female ratio was 1:1.9. In another study, the authors found that 75% of their patients were male and 25% were female. This finding is different from the findings of the present study and could probably be attributed to selection bias.[10]

Majority of cases were outdoor patients (62.0%) as compared to indoor patients (38.0%). Maximum number of cases were from Orthopaedics (26.9%) followed by Surgery (24.1%). For all these departments, greater number of cases were from OPD than IPD except Orthopaedics where more number of patients were from IPD. The preponderance of cases in Orthopaedics and Surgery departments could be due to the fact that maximum number of Staphylococcus aureus was isolated from pus/swabs samples which were collected from these two departments. 57.4% of Staphylococcus aureus isolations were from pus/swabs. Other authors also reported maximum isolation from pus/wound swabs.[10]

In the present study, 29.6% of Staphylococcus aureus was MRSA. As far as MRSA is concerned different studies have reported different findings. In a pilot programme of MRSA surveillance in India, 26.7% of Staphylococcus aureus strains were found to be MRSA in Mumbai, 47.1% in Bangalore and 42.5% in New Delhi.[11] Other authors have reported 79.6% of MRSA isolation from Hyderabad,[12] 56.0% from Vadodara,[13] 31.2% from South India,[14] 34.2% from Uttarakhand[15] and 39.1% from Kolkata.[16]

The lower rate of MRSA isolation in our region is probably due to the fact that this study was conducted in a rural setting with patients having less exposure to antibiotics.

By the agar dilution method, 10.2% of Staphylococcus aureus were VISA, 3.7% were VRSA and 1.9% were LRSA. The disc diffusion method; however, detected more number of VRSA 8.3% cases and LRSA 3.7%, which were probably false positives.

A 10-year-old study; however, reported that only 0.77% of their Staphylococcus aureus strains were VISA and 0.26%

were VRSA.[17] These findings are very low as compared to the present study probably because of the time lapse between these studies. Other authors have reported VISA isolations in 4.47% and VRSA in 1.96% of Staphylococcus aureus from Hyderabad.[12] Similar findings were also reported from Vadodara (1.02%) and Kolkata (1.43%).[13,16]

On the other hand, very high rate of LRSA isolation was reported from Nagpur, in which 23.5% of their Staphylococcus aureus strains were LRSA as compared to 1.9% in the present study.[7]

Notably all VRSA and LRSA were isolated from indoor patients, none being community associated, a clear indication that these strains develop in hospital settings under high antibiotic pressure.

All VRSA and VISA strains were resistant to amoxicillin, cefotaxime, cefoxitin and cefuroxime. All VISA strains were sensitive to linezolid and all VRSA strains were sensitive to imipenem. The two strains of LRSA were resistant to amoxicillin, cefotaxime, cefoxitin, cefuroxime, teicoplanin and vancomycin and sensitive to amikacin and imipenem.

CONCLUSION

VRSA and LRSA in this region was found to be relatively low (3.7% and 1.9% respectively of all Staphylococcus aureus isolates) as compared to other part of India, which is probably because a large number of the subjects in this study came from rural areas where the organisms are not exposed to as much antibiotic pressure as in the urban areas. Determination of MIC was found to be a better method for detection of VRSA and LRSA. Though it is cumbersome to perform the MIC, it is recommended that this test be performed for detection of vancomycin and linezolid resistance. Smaller laboratories can perform a screening test using vancomycin agar screen test using 6 µg/mL of vancomycin in brain heart infusion agar; if a strain grows on this medium indicating resistance, further tests could be put up.

The number of LRSA, though low, is a cause for concern underlining the importance of performing culture and sensitivity in infectious cases in the hospital instead of putting patients on empirical treatment which leads to increase of resistant strains. This will prevent random use of drugs like vancomycin and linezolid keeping them in reserve for those cases where all other drugs are resistant.

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