Research Paper Medical Science

Linezolid – Losing Its Golden Touch To A Golden Bacteria

Dr. Dibyendu Banerjee	Associate Professor. Calcutta National Medical College. Department - Department of Microbiology. City - Kolkata. State – West Bengal. Country – India.
Dr. Baishali Chakraborty	Assistant Professor. Institution - Calcutta Medical College (Medical College, Kolkata). Department - Department of Microbiology. City - Kolkata. State – West Bengal. Country – India.
Dr. Banya Chakraborty	Professor. Institution - Calcutta Medical College (Medical College, Kolkata). Department - Department of Microbiology. City - Kolkata. State – West Bengal. Country – India.

ABSTRACT

Background: Staphylococcus aureus is a very common and dangerous pathogen, causing pyogenic infections all over the world. This pathogen by becoming methicillin resistant (MRSA) became a cause of major concern to the medical fraternity. A chief alternative in the hand of clinicians is linezolid – an oxazolidinone.

Materials and Methods: Cultures from100 wound swabs revealed 66 Staphylococcus aureus isolates. Among them, 4 Staphylococcus aureus showed resistance to Linezolid by disc diffusion method. They were found to be methicillin resistant as well. Then PCR of these 4 resistant strains as well as a sensitive strain was done followed by DNA sequencing.

Results: Sequencing of the domain V region of the 23S rRNA gene revealed the presence of a G2576U mutation in the LRSA (LZD-resistant S. aureus) isolates in two 23s rRNA copy, rrn1 & rrn5.

Conclusions: We found a 6% incidence of LRSA, which were also MRSA, thus seriously jeopardizing treatment options. LRSA is a rare occurrence ,and rarely reported from Indian subcontinent. A constant vigil is necessary to detect this resistance ,if possible with tools of molecular epidemiology.

KEYWORDS : Staphylococcus aureus, Linezolid resistance, PCR

INTRODUCTION

Staphylococcus aureus, a well-known pathogen producing golden colonies on Blood agar, is notorious for causing pyogenic infections, both in the community setup, as well as in hospital environment. It is also remarkable for developing multidrug resistance day by day – especially becoming methicillin resistant *Staphylococcus aureus*, otherwise known as MRSA. Linezolid, an oxazolidinone, has shown potent activity against gm-positive organisms like MRSA, methicillin resistant coagulase negative Staphylococci (MR-CoNS), vancomycin resistant enterococci (VRE), and multidrug resistant *Streptococcus pneumoniae*, and has thus become the clinicians' favourite blue-eyed boy. [1] It uniquely inhibits protein synthesis by binding to the peptidyltransferase center (PTC) of the 50S ribosomal subunit. [2,3]

Linezolid resistance in *S. aureus* is extremely uncommon, and the surveys have revealed that >99% isolates are susceptible. [4,5] There are 3 main mechanisms for developing LZD resitance:

1. Mutations the domain V region of one or more of the 5 copies of 23S rRNA gene.
2. Acquisition of plsmid medated ribosoalmethytransferase cfr gene.
3. Deletion/mutation in the ribosomal protein L3 of PTC.

Among these three mechanisms, LZD resistance has been largely attributed to the first one. [3]

MATERIALS AND METHODS

We examined 100 nonrepetitive pus samples from patients admitted in surgery wards. Out of these 100 samples, pure growth of *Staphylococcus aureus* was found in 66 cases, and were confirmed by colony characters, Gram staining, Tube coagulase and DNAse tests. *S aureus* ATCC 25923 strain was used as a control.

Susceptibility testing was performed by Kirby-Bauer disc diffusion method following CLSI guideline. [6] The *Staphylococcus aureus* isolates were tested against a panel of antibiotics comprising of vancomycin, cotrimoxazole, amikacin, ofloxacin, penicillin, erythromycin, cefoxitin and linezolid. Out of 66 S aureus isolates 4 isolates showed growth upto the edge of 30 mg linezolid disc. Linezolid susceptibility was then repeated by Stokes method with *Staphylococcus aureus* ATCC strain as control, and again the 4 test strains showed resistance. They showed resistance to cefoxitin as well, and hence were considered to be methicillin resistant *S aureus* (MRSA) .

These 4 isolates were preserved by stabbing it in a semisolid media. At the same time a linezolid sensitive isolate of *Staphylococcus aureus* was also preserved.

DNA extraction was done from all the 5 (4 suspected LRSA and 1 linezolid sensitive) isolates and PCR was done with Taq DNA polymerase.

Then, following Agarose gel electrophoresis of the amplified product, DNA sequencing was done from lanes 2,3,4 and 6 of gel (lane 2 LNZ rrn 1 & ;ane 6 LNZ rrn 5).

After sequencing we got the following three sequences of two different copy of 23s rDNA (LNZr1, LNZr5) and as control we used PCR product of LSSA (LZD-sensitive *S. aureus*) DNA with rDNA1 primer set.

Sequencing of the domain V region of the 23S rRNA gene from the 4 LRSA isolates revealed the presence of a G2576U mutation (*Escherichia coli* numbering) in the LRSA (LZD-resistant *S. aureus*) isolates in comparison to LSSA (LZD-sensitive *S. aureus*) isolate in two 23s rRNA copy, rrn1 & rrn5. However, we didn't find any other reported mutation like G2447U, G2505A, C2512U, G2513U, and C2610G.

DISCUSSION

Linezolid resistance, thankfully, is an extremely rare occurrence. The Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) study, 2007 for linezolid resistance came up with an overall resistance rate to linezolid in 23 countries to 0.03%. [5] A similar worldwide programme, LEADER 2009, which monitors and tracks linezolid resistance in USA since 2004, reported the resistance rate to be 0.34%. [7] In our study, we found 6% LRSA , which were MRSA also.

Data on linezolid resistance of Staphylococcus from Asian countries, especially Indian subcontinent is extremely rare and whatever scarce reports are found – DNA sequencing to point out the type of point mutation / mechanism of resistance has not been performed in almost all the cases. Linezolid resistant Coagulase negative Staphylococcal sepsis has been reports by performing E-test. [8] The strains of *S.aureus* found by us were unique in the sense that they were MRSA strains showing resistance to linezolid, hence compromising almost all the avenues of treatment.The microbiology laboratory in every hospital should keep a careful vigil to detect this variety of the golden pigment producing bacteria, and should preferably identify it with molecular tools to define the type of mutation, which would help in epidemiological study as well..

REFERENCES

1. Diekema DJ, Jones RN. Oxazolidinone antibiotics. Lancet 2001;358:1975-82.
2. Swaney SM, Aoki H, Ganoza MC, Shinabager DL. The oxazolidinoe linezolidinhibits initiation of protein synthesis in bacteria. Antimicrob Agents Chemother 1998;42;3251-5.
3. Pillai SK, Sakoulas G, Wennersten C, *et al*. Linezolid resistance in Staphylococcus aureus: Characterization and stability of resistant phenotype. J Inf Dis 2002;186:1603-7.
4. Jones RN, Fritsche TR, Sader HS, Ross JE. LEADER surveillance program results for 2006: an activity and spectrum analysis of linezolid using clinical isolates from the United States (50 medical centers). Diagn Microbiol Infect Dis 2007;59:309-17.
5. Jones RN, Kohno S, Ono Y, Ros JE, Yanagihara K. ZAAPS International Surveillance Program (2007) for linezolid resistance: Results from 5591 Gram-positive clinical isolates in 23 countries. Diagn Microbiol Infect Dis 2009;64:191-201.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. M100-S16. Wayne, PA: CLSI;2006.
7. Farell DJ, Mendes RE, Ross JE, Sader HS, Jones RN. LEADER Program Results for 2009: An Activity and Spectrum Analysis of Linezolid Using 6,414 Clinical Isolates from 56 Medical Centers in the United States. Antimicrob Agents Chemother 2011;55:3684-90.
8. Peer MA, Naseer RA, Kakru DK, Fomda BA, Bashir G, Sheikh IA. Sepsis due to Linezolid resistant Staphylococcus kohnii and Staphylococcus kloosi; First reports of linezolid resistance in coagulase negative staphylococci in India. Indian Journal of Medical Microbiology 2011; 29(1): 60-70.