ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

LINEZOLID RESISTANCE IN COAGULASE NEGATIVE STAPHYLOCOCCUS IN CRITICAL CARE UNITS OF A TERTIARY CARE HOSPITAL IN EASTERN INDIA: A CROSS SECTIONAL STUDY.

Microbiology

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ABSTRACT

Linezolid is one of the limited antimicrobials to treat multidrug resistant gram positive bacteria. The linezolid resistance is less common but emerging. Coagulase negative staphylococcus is now also common and significant pathogen in bloodstream infection. Linezolid resistant Coagulase negative staphylocoocus are emerging and causes fatal infection among the intensive care unit patients. In our study we demonstrate the frequency of linezolid resistance among Coagulase negative staphylocoocus isolated from intensive care unit patients'bloodstream infection. The MIC of Linezolid ranged from 8 to 32µg/ml. LR was observed in three different CoNS species.Our study emphasises on strict surveillance of emerging drug resistance in hospital setting .

KEYWORDS

Coagulase Negative Staphylococus, Linezolid, Resistance

INTRODUCTION:

Linezolid is an oxazolidinone antimicrobial approved for clinical use in 2000. Linezolid inhibits initiation of bacterial protein synthesis by preventing the formation of the tRNA–ribosome–fMet–mRNA tertiary complex.[1] Linezolid is active against the majority of clinically relevant Gram-positive cocci, including methicillin-resistant staphylococci (MRSA & MRCoNS), Vancomycin-resistant enterococci and Penicillin-resistant streptococci[2].

Coagulase-negative staphylococci (CoNS) are common colonizers of the human skin and are the most frequent constituent of the normal flora at this site. Although once considered to be non-virulent and probably contaminant microbes, these organisms have become increasingly recognized as agents of clinically significant nosocomial infections.[3]

Despite a decade of its clinical use, resistance to linezolid has remained stable and extremely low with only sporadic cases being reported. Linezolid resistance in coagulase negative staphylococci has been reported rarely from India. In the current study, we studied the clinical, demographic profile & antibiotic susceptibility pattern of 15 isolates of coagulase-negative staphylococci with Linezolid resistance causing blood stream infections that were recovered from intensive care unit from a tertiary care hospital in Kolkata.

MATERIALS & METHODS:

In a period of 13 months (July2018-August 2019) 15 isolates of Coagulase Negative (out of Total 406) Staphylococci causing blood stream infection showed Linezolid resistance by modified Kirby Bauer disc diffusion method. All the isolates were recovered in duplicate from blood culture set. CoNS were identified by Gram stain, the presence of catalase, and negative tube coagulase test Species identification and antibiotic susceptibity testing was then performed with the Vitek 2 compact system (bioMe´rieux) & MICs were determined. The Clinical and Laboratory Standards Institute recommendations were used for antimicrobial susceptibility testing interpretation (CLSI, 2017). S. aureus ATCC 25923 and ATCC 29213 were used as quality control strains. Demographic data, clinical history including prior antibiotic therapy, invasive procedures, and co-morbid conditions were obtainedfrom patients; records. Isolates were classified as nosocomial as the sample was collected more than 48 h following admission to the hospital. Sepsis related Organ failure Assessment Score(SOFA) were determined for all the patients using android app QxMD Medical Software Inc.

RESULTS:

Out of total 406 ConS isolated during the study period 286 were Methicillin resistant (MRCoNS) and Fifteen (15) were Linezolid-resistant which were also MRCoNS isolates, including nine *Staphylococcus haemolyticus*, and four *Staphylococcus cohnii subspecies urealyticus*, two *Staphylococcus epidermidis* were isolated from patients suffering blood stream infections. Eleven patients were admitted in the critical care unit, two patient were admitted in Neuro ICU and Pediatric ICU each. None of the patients received Linezolid previously. All of the patients developed this infection after admission in the hospital. The MIC for Linezolid was in the range of >8µg/ml to > 32 µg/ml on all the occasions. The patient's outcome correlated significantly with SOFA score. As higher the SOFA score higher were the mortality.

In our study we identified the fifteen linezolid resistance isolates by breakpoint of >8 µg/ml in the VITEK 2 COMPACT System and further tried to estimate their MICs by Etest method and only 2 isolates were found to have MICs >32 µg/ml.

Only one of the isolates showed simultaneous resistance to Vancomycin, but rest were susceptible to Vancomycin. None of the isolates showed Inducible Clindamycin resistance. All the isolates were resistant to Ciprofloxacin, Erythromycin and Clindamycin. All the isolates were susceptible to Daptomycin and Tetracycline. Seven patients had fatal outcomes whereas rest Eight patients survived. The clinical & demographic profiles of the patients are shown in table 1 and the antibiotic susceptibility pattern in Table 2.

DISCUSSION :

Though CoNS were frequently isolated from blood cultures, but their role as pathogens were questioned and they were considered as contaminants. In recent years, in nosocomial setting, prolonged hospital stay, frequent interventions and use of intravascular devices, coagulase negative staphylococci are emerging as important pathogens capable of causing bloodstream infections (BSI).Stringent asepsis during collection, repeated isolation and proper identification can justify the role of CoNS as true pathogens.

Data collected in medical intensive care units through the National Nosocomial Infections Surveillance (NNIS) system between 1992 and 1997 revealed that CoNS accounted for 36% of all bloodstream isolates, making these organisms the most common cause of nosocomial bloodstream infections.[4] A second database of nosocomial bloodstream infections in United States hospitals from 1995 to 2002 also identified CoNS as the most common cause, responsible for 31% of cases.[5] Recently, in a study conducted by All India Institute of Medical Sciences, over a period of 30 months, showed out of 469 staphylococcal bacteraemia cases, 47% (221/469) of the isolates were found to be CoNS.[6]

Staphylococcus cohnii is a microorganism belonging to the CoNS group. It is composed of two major subspecies: *Staphylococcus cohnii* subsp. *cohnii* and *Staphylococcus cohnii* subsp. *urealyticus*. They are nonmotile, nonspore-forming, Gram-positive, coagulase negative, aerobic, clustering cocci that produce catalase. It is occasionally a skin colonizer. It may be responsible for bacteremia relating to catheters,

surgical prostheses, brain abscess, endocarditis, pneumonia, urinary tract infection and septic arthritis, generally presenting with a multiresistant profile in human infections, with nearly 90% of them showing resistance to methicillin.[7] A Brazilian survey on blood samples positive for CoNS in a nosocomial setting showed that the prevalence of *Staphylococcus cohnii* was 5.9% [8] A Greek study reported an outbreak of infection caused by linezolid-resistant *Staphylococcus cohnii* subsp. *urealyticus* in a intensive care unit, with septicemia in four patients; this was due to selection pressure through linezolid use in case of vancomycin-resistant *Enterococcus faecium*.[9] Among coagulase-negative staphylococci (CoNS), Staphylococcus haemolyticus is the second most frequently isolated from human blood cultures and has the highest level of antimicrobial resistance [10] In a study on Coagulase negative Staphylococci causing blood-stream infections from India, The majority of CoNS isolated were constituted by *Staphylococcus haemolyticus* (47.5%) followed by *Staphylococcus epidermidis* (33.9%), *Staphylococcus hominis* (11.86%), *Staphylococcus cohnii* (5.08%) and *Staphylococcus warneri* (1.69%).[11]

Linezolid, with both oral and parenteral formulations, is a very important therapeutic option shown to be effective against Gram positive isolates, especially MRSA and MRCoNS. Data from the USA and global surveillance studies report, 1% of S. aureus and 2% of CoNS are linezolid resistant.[12, 13] Recently, linezolid-resistant staphylococci have become an increasing problem, with few outbreaks being reported from European countries and the USA. [9,14]Data on linezolid resistance from Asian countries are scarce. Linezolid resistant MRCoNS are reported in a study from China.[15] there are very few reports of Linezolid resistant Staphylococcus strains from India. Blood-stream infection due to Linezolid-resistant *Staphylococcus cohnii* and *Staphylococcus kloosii* are reported from Kashmir, India.[16] Interestingly in our study unlike any other study we found there was no prior Linezolid exposure in any of our patients, it signifies there is a rapid method of resistance transfer between the local bacterial population acquired from other sources.[17]We came along way after reporting first few cases in India in 2011[18] to today where Linezolid resistance is well established and getting rampant. As our study described all the LRCoNS isolates are from critical care unit and 7 of the 9 are are from same unit we suspect Horizontal spread of resistant isolates among diverse species were the probable mechanisms of transmission. Characterization of the resistance mutations in clinical isolates may be helpful in efforts to design the next generation of oxazolidinones and contain the outbreak.

CONCLUSION:

Emerging resistance to linezolid is a matter of great concern in context of the ever increasing multi-resistant gram-positive cocci and the relatively limited treatment options for the infections caused by these organisms, oxazolidinones are likely to remain an important part of our antimicrobial armamentarium. To avoid spread of staphylococcal resistance in ICUs, we suggest measures such as proper hand hygiene and adequate care in central venous catheter handling should be taken, and policies regarding antimicrobial drug use must be applied.

Table-1 Clinical characteristics of patients with linezolid-resistant MRCoNS

Isolate	Ward	Antibiotic Exposure	Age	Sex	Linezolid MIC	Underelying Condition	SOFA SCORE	Outcome
Staphylococcus haemolyticus (LRS1)	CCU	Colistin	38 y	Male	> 8	C4-C7 laminectomy for evacuation of extradural hematoma	15	Died
Staphylococcus cohnii subspecies urealyticus (LRS2)	CCU	Cefoperazone-sulbactum	21y	Male	>8	Myesthenia gravis ,put on ventilator	9	Survived
Staphylococcus haemolyticus (LRS3)	CCU	Meropenem, Piperacillin-tazobactum	40 y	Male	>8	Quadruplegia due to attempt of Hanging	11	Died
Staphylococcus haemolyticus (LRS4)	Neuro ICU	Colistin, Piperacillin-tazobactum	62 y	Female	>8	Meningioma	20	Died
Staphylococcus cohnii subspecies urealyticus (LRS5)	CCU	Piperacillin-tazobactum, meropenem	43y	Male	>8	Endocarditis	12	Survived
Staphylococcus haemolyticus (LRS6)	CCU	Ceftriaxone, Gentamicin	52y	Male	>32	Left heart Failure	10	Survived
Staphylococcus haemolyticus (LRS7)	CCU	Piperacillin-Tazobactum	56y	Male	>8	Endocarditis	8	Survived
Staphylococcus cohnii subspecies urealyticus (LRS8)	CCU	Colistin	65y	Female	>32	CVA	11	Survived
Staphylococcus epidermidis (LRS9)	PICU	Ceftriaxone, Gentamicin	9 months	Male	>8	Status epilepticus	10	Survived
Staphylococcus haemolyticus (LRS10)	CCU	Colistin	39 y	Male	> 8	Intestinal Perforation with septic shock	20	Died
Staphylococcus cohnii subspecies urealyticus (LRS11)	CCU	Cefoperazone-sulbactum	27y	Male	>8	Hepatic abscess	14	Survived
Staphylococcus haemolyticus (LRS12)	CCU	Meropenem, Piperacillin-tazobactum	50 y	Male	>8	Head injury with GCS 2	22	Died
Staphylococcus haemolyticus (LRS13)	Neuro ICU	Colistin, Piperacillin-tazobactum	62 y	Female	>8	Snake bite	18	Died
Staphylococcus cohnii subspecies urealyticus (LRS14)	CCU	Piperacillin-tazobactum, meropenem	48y	Male	>8	Diabetes Mellitus with chronic liver disease	14	Survived
Staphylococcus haemolyticus (LRS15)	PICU	Ceftriaxone, Gentamicin	2y	Male	>8	VSD, pneumonia	10	Survived

Table- 2 Table showing antibiotic susceptibility pattern of Linezolid-Resistant MRCoNS

Antibiotics	MIC (µg/ml)														
	1*	2	3*	4*	5	6	7	8*	9	10*	11	12*	13*	14	15
Benzylpenicillin	≥.5(R)	≥.5(R)	≥.5 (R)	≥.5(R)	≥.5(R)	≥.5(R)	≥.5(R)	≥.5(R)	≥.5(R)	≥.5(R)	≥.5(R)	≥.5 (R)	≥.5(R)	≥.5(R)	≥.5(R)
Oxacillin	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)
Gentamicin	4(S)	4(S)	≥16(R)	8 (I)	8(I)	≤.5(S)	4(S)	≥16(R)	≥16(R)	4(S)	4(S)	≥16(R)	8 (I)	8(I)	≤.5(S)

Ciprofloxacin	≥8(R)	≥8(R)	≥8(R)	≥8 (R)	≥8(R)	≥8(R)	≥4(R)	≥8(R)	4(R)	≥8(R)	≥8(R)	≥8(R)	≥8 (R)	≥8(R)	≥8(R)
Levofloxacin	4(R)	≥8(R)	4 (R)	≥8(R)	≥8(R)	≤1(S)	≥8(R)	4(R)	4(R)	4(R)	≥8(R)	4 (R)	≥8(R)	≥8(R)	≤1(S)
Erythromicin	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)	≥8(R)
Clindamycin	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥8(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)	≥4(R)
Vancomycin	1(S)	1(S)	1 (S)	1(S)	1(S)	**≥32(R)**	1(S)	1(S)	1(S)	1(S)	1(S)	1 (S)	1(S)	1(S)	1(S)
Teicoplanin	2(S)	2(S)	4(S)	4(S)	≤.5(S)	**≥32(R)**	2(S)	4(S)	2(S)	2(S)	2(S)	4(S)	4(S)	≤.5(S)	2(S)
Linezolid	**≥8(R)**	**≥8(R)**	**≥8(R)**	**≥8(R)**	**≥8(R)**	**≥32(R)**	**≥8(R)**	**≥32®**	**≥8(R)**	**≥8(R)**	**≥8(R)**	**≥8(R)**	**≥8(R)**	**≥8(R)**	**≥8(R)**
Tetracycline	2(S)	≤1(S)	2(S)	2(S)	≤1(S)	≥16(R)	2(S)	≤1(S)	≤1(S)	2(S)	≤1(S)	2(S)	2(S)	≤1(S)	≥16(R)
Trimethoprim-Sulfamethoxazole	≥320 (S)	≥320(R)	≥320(R)	≥320(R)	≥320(R)	≤10(S)	≥320(R)	≥320(R)	40(S)	≥320(S)	≥320(R)	≥320(R)	≥320(R)	≥320(R)	≤10(S)
Daptomycin	.25(S)	.5(S)	1(S)	.25(S)	.25(S)	1(S)	1(S)	.25(S)	.25(S)	.25(S)	.5(S)	1(S)	.25(S)	.25(S)	1(S)
Tigecycline	≤.12(S)	≤.12(S)	.25(S)	.5(S)	≤.12(S)	.25(S)	.25(S)	.5(S)	≤.12(S)	≤.12(S)	≤.12(S)	.25(S)	.5(S)	≤.12(S)	.25(S)

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