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ORIGINAL ARTICLE

Antimicrobial susceptibility patterns of Linezolid-resistant coagulase-negative staphylococci in Pakistan

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ABSTRACT

OBJECTIVE: To determine the antimicrobial resistance pattern in Linezolid-resistant coagulase negative staphylococcus (CoNS) isolates in Pakistan.

METHODS: This cross-sectional study was conducted from February and July 2022, at a private laboratory in Lahore, with samples collected from various regions across Pakistan. The study included microbiological samples such as blood and wound specimens, from which 4153 isolates of CoNS were identified. Of these, only 64 isolates that were both coagulase-negative and Linezolid-resistant were selected for further investigation. The antimicrobial susceptibility patterns of these Linezolid-resistant isolates were analyzed. The antibiotics tested included Amikacin, Ciprofloxacin, Clindamycin, Doxycycline, Erythromycin, Fusidic Acid, Gentamicin, Oxacillin, Teicoplanin, Trimethoprim-Sulfamethoxazole, And Vancomycin. Data analysis was performed using SPSS version-22.

RESULTS: Among 4153 samples, 64 (1.54%) were Linezolid-resistant CoNS isolates, predominantly from adults (aging 18-59 years) and senior adults (aging >59 years), with a higher prevalence in males (56.3%). Resistance was observed in 51.6% of blood and 48.4% of pus samples. Most isolates were also resistant to Teicoplanin (100%), Ciprofloxacin (92.2%), Oxacillin (89.1%), And Fusidic Acid (84.4%), While Sensitivity Was Highest For Vancomycin (85.9%) And Doxycycline (79.7%). Statistically significant differences were noted for all antibiotics except erythromycin.

CONCLUSION: Our findings highlight the importance of monitoring the evolution of Linezolid resistance in CoNS as Linezolid resistant isolates also showed high resistance to some other major antimicrobial drugs (e.g. Teicoplanin, Ciprofloxacin, Oxacillin etc.). Linezolid resistance must be closely monitored, especially when frequent and prolonged Linezolid therapy is indicated to implement control measures and reduce the risk of CoNS spreading in the community.

KEYWORDS: Linezolid (MeSH); Multidrug Resistance (MeSH); Drug Resistance, Microbial (MeSH); Drug Resistances Microbial (MeSH); Antimicrobial drug resistance (Non-MeSH); Microbial Sensitivity Test (MeSH); MeSH); Coagulase (MeSH); Staphylococcal Infections(MeSH); Bloodstream Infection (Non-MeSH): Vancomycin (MeSH); Infections, Nosocomial (MeSH); Cross Infection (MeSH).

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INTRODUCTION

Coagulase negative Staphylococci (CoNS) are a significant component of the normal cutaneous and mucous flora. However, with the increasing use of implanted medical devices and procedure-related changes, they have become one of the leading causes of nosocomial infections.[1] Managing CoNS infections is challenging, as these isolates often show resistance to various antimicrobials, particularly Penicillins and Oxacillin/Methicillin.[2]

Methicillin-resistant Staphylococcus aureus (MRSA) and CoNS are among the Gram-positive bacteria effectively treated by the Oxazolidinone antibiotic, Linezolid.[3] Despite Linezolid being a cornerstone in the treatment of multidrug-resistant Gram-positive infections, resistance to this drug has emerged.[4] It works by binding to the 50S ribosomal subunit, preventing the assembly of the initiation complex and thereby inhibiting protein synthesis with its bacteriostatic properties.[5] Linezolid resistance is often linked to mutations in domain V of 23rRNA with G2576T substitution and acquisition of methyltransferase gene *cfr*.[6,7]

Linezolid is a very desirable antibiotic for the therapy of possible or proven staphylococcal infections due to its great oral absorption.[8] However, the earliest Linezolid resistance strain was claimed in 2001 in the USA.[6] Since then, Linezolid resistant strains start to appear worldwide. In many regions such as North America, Ireland, China, Brazil, Greece, Spain, Italy and France, Linezolid resistance in CoNS infections has been reported.[2] In Pakistan, a tertiary care cardiac facility reported the earliest case of coagulase-negative, Linezolid-resistant Staphylococcus in 2018.[9] There is relatively little research done in Pakistan, despite the fact that Linezolid resistance in CoNS infections constitutes a significant health risk.

METHODS

This cross-sectional study was conducted from February 2022 to July 2022 in a private laboratory in Lahore, which received samples from various regions across Pakistan. Ethical approval for the study was granted on February 3, 2022, with IRB number CIP/IRB/1099. The study included 4153 isolates of CoNS derived from

microbiological samples such as blood and wound cultures. CoNS were identified based on positive gram staining (cocci in clusters) and catalase tests, with negative tube coagulase tests. Among these, only 64 isolates that were resistant to Linezolid were included in this study, while CoNS isolates that were Linezolid-sensitive were excluded.

The isolates were further identified and characterized using MALDI-TOF and Vitek [2] techniques. Antimicrobial susceptibility was assessed using the agar dilution method and disc diffusion method. Minimal inhibitory concentrations (MICs) were determined using the E test and Vitek. Staphylococcus aureus ATCC 25923 was used as a quality control for susceptibility testing, following the Clinical Laboratory Standards Institute (CLSI) guidelines.[10] The study evaluated susceptibility to Linezolid, Amikacin, Ciprofloxacin, Clindamycin, Doxycycline, Erythromycin, Fusidic Acid, Gentamicin, Oxacillin, Teicoplanin, Trimethoprim-Sulfamethoxazole, And Vancomycin.

Data were entered and analyzed using SPSS version 22. The binomial test of proportion was employed to detect statistical differences between resistant and sensitive isolates, with statistical significance defined as a p-value ≤0.05.

RESULTS

Among the 4153 samples, 64 (1.54%) were Linezolid resistant. Among those 64 Linezolid resistant CoNS isolates, it was observed that most of the isolates were of adult patients followed by senior adults. The resistance of Linezolid was relatively more common among male patients. Out of those 64 samples, Linezolid resistance was observed in 51.6% blood samples followed by 48.4 % pus samples (Table I).

Antimicrobial sensitivity patterns showed that Linezolid-resistant isolates also demonstrated resistance to Teicoplanin. About 54 (84.4%) isolates were resistant to Fusidic acid, 59 (92.2%) were resistant to Ciprofloxacin and 57 (89.1%) were resistant to Oxacillin. However, most of the isolates were sensitive to Vancomycin followed by Doxycycline as shown in Table II.

Table I: Baseline features of patients with Linezolid resistant isolate

Variables		Frequency	Percentage
Age (years)	<12 (Children)	11	17.2
	12-17 (Adolescents)	02	3-1
	18-59 (Adults)	34	53.1
	> 59 (Senior Adults)	17	26.6
Gender	Male	36	56.3
	Female	28	43.8
Location	Punjab	51	79.7
	Sindh	05	7.8
	Khyber Pakhtunkhwa	08	12.5
Specimen	Blood	33	51.6
	Wound	31	48.4

The binomial test of proportion was employed to assess the statistical differences between resistant and sensitive isolates. Statistically significant differences were observed among the resistant and sensitive isolates for Amikacin, Ciprofloxacin, Clindamycin, Doxycycline, Fusidic Acid, Gentamicin, Oxacillin, Teicoplanin, Trimethoprim-Sulfamethoxazole, and Vancomycin. However, at the 5% level of significance, no statistically significant difference was found between Erythromycin-resistant and Erythromycin-sensitive isolates.

Binomial test of proportion was used to observe the statistical difference between resistant and sensitive isolates. Among resistant and sensitive isolates of Amikacin, Ciprofloxacin, Clindamycin, Doxycycline, Fusidic Acid, Gentamicin, Oxacillin, Teicoplanin, Trimethoprim Sulfamethoxazole and Vancomycin; statistically significant difference was demonstrated. At the 5% level of significance, there was no statistically significant difference between the

Table II: Antimicrobial sensitivity pattern among Linezolid resistant isolates

Antibiotics	Resistant	Sensitive	p-value
Amikacin	17 (26.6%)	47 (73.4%)	0.000*
Ciprofloxacin	59 (92.2%)	5 (7.8%)	0.000*
Clindamycin	47 (73.4%)	17 (26.6%)	0.000*
Doxycycline	13 (20.3%)	51 (79.7%)	0.000*
Erythromycin	36 (56.3%)	28 (43.8%)	0.382
Fusidic Acid	54 (84.4%)	10 (15.6%)	0.000*
Gentamicin	47 (73.4%)	17 (26.6%)	0.000*
Oxacillin	57 (89.1%)	7 (10.9%)	0.000*
Teicoplanin	64 (100.0%)	0 (00.0%)	0.000*
Trimethopr Sulfamethoxazole	46 (71.9%)	18 (28.1%)	0.001*
Vancomycin	9 (14.1%)	55 (85.9%)	0.000*

*Statistically Significant Difference i.e. p-value ≤ 0.05

Erythromycin isolates that were resistant and those that were sensitive.

DISCUSSION

In this study on 4153 samples, 64 (1.54%) were linezolid-resistant CoNS, predominantly affecting males (56.3%) and adults aged 18-59 years and over 59 years. Resistance was noted in 51.6% of blood and 48.4% of pus samples. The isolates were universally resistant to Teicoplanin (100%), highly resistant to Ciprofloxacin (92.2%), Oxacillin (89.1%), and Fusidic acid (84.4%), while showing higher sensitivity to Vancomycin (85.9%) and Doxycycline (79.7%). Statistically significant resistance differences were observed for all antibiotics tested except Erythromycin.

Many researchers have highlighted the growing significance of identifying CoNS as they are increasingly recognized as key pathogens in healthcare-associated infections.[11] The antibiotic-resistance profiles of CoNS isolates were examined in this study. Reports from clinical samples of hospitalized patients have indicated resistance to several critical antimicrobial agents, including Linezolid. While over 98% of Staphylococcus strains remain susceptible to Linezolid, resistance has been documented in 1.4% of coagulase-negative Staphylococcus isolates (n = 73/5202).[12] A large multicenter study conducted in Greece from 2011 to 2013 showed a notable increase in Linezolid resistance among CoNS, rising from 6.9% to 9%.[13] In our study, 1.54% of CoNS isolates (n=64/4153) were resistant to Linezolid, which may reflect an increased resistance rate linked to the clinicians' preference for this antibiotic.

Recent studies have highlighted an increase in the isolation rates of CoNS from bloodstream infections. For instance, out of 581 CoNS isolates from a hospital in Pakistan, 311 (53.5%) were from blood samples, and 204 (35.1%) were from pus/swabs.[14] Another study reported that bloodstream infections accounted for 51% to 78% of CoNS infections among newborns with very low birth weights.[15] In our study, 51.6% of CoNS isolates were from blood, while 48.4% were from wounds.

A study conducted in Jordan assessed the prevalence and antimicrobial susceptibility of CoNS isolates from clinical specimens. According to CLSI 2009 criteria, CoNS isolates exhibited high sensitivity to Vancomycin, Linezolid, Rifampin, and Nitrofurantoin but showed significant resistance to Penicillin, Ampicillin, and other antibiotics. [16] Another study from India in 2019 reported that all Linezolid-resistant CoNS isolates were also resistant to Erythromycin, Cefoxitin, Clindamycin, and Trimethoprim/Sulfamethoxazole, with 80% resistant to Gentamicin and 90% resistant to Chloramphenicol and Ciprofloxacin. While all isolates were susceptible to Vancomycin (MIC range: 1-4 μg/ml), only one-third were susceptible to Teicoplanin. [17] In our study, linezolid-resistant strains were also resistant to Teicoplanin, and we observed high resistance to Ciprofloxacin, Fusidic Acid, Oxacillin, and Trimethoprim-sulfamethoxazole, with sensitivity noted for Vancomycin, Amikacin, and Doxycycline. The ongoing development of antibiotic resistance highlights the importance of selecting appropriate antimicrobial therapies.

A surveillance study in Italy from January 2016 to October 2018 evaluated the susceptibility of 828 CoNS isolates and found no resistance to Teicoplanin, Vancomycin, or Linezolid. However, varying resistance levels were observed for other antibiotics, including Ampicillin (87%), Penicillin (86%), Oxacillin (70%), Erythromycin (69%), Ciprofloxacin (54%), Gentamicin (47%), Trimethoprim-Sulfamethoxazole (30%), and Clindamycin (28%).[18] In our study, of the 4153 CoNS isolates analyzed, 64 (1.54%) were identified as Linezolid-resistant.

CONCLUSION

Our findings emphasize the critical need to monitor the development of Linezolid resistance in CoNS. Linezolid-resistant isolates have also demonstrated significant resistance to other key antimicrobial agents, such as Teicoplanin, Ciprofloxacin, and Oxacillin. It is essential to closely monitor Linezolid resistance, particularly when prolonged or frequent Linezolid therapy is required. This vigilance will help implement effective control measures and minimize the risk of resistant CoNS spreading within the community.

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AUTHORS' CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

AS: Study design, acquisition of data, critical review, approval of the final version to be published

NM: Acquisition and analysis of data, drafting the manuscript, approval of the final version to be published

ZR: Concept and study design, analysis and interpretation of data, critical review, approval of the final version to be published

IY: Concept and study design, critical review, approval of the final version to be published

RH: Conception, drafting the manuscript, critical review, approval of the final version to be published

SJ: Acquisition of data, drafting the manuscript, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

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