

Constitutive and Inducible Clindamycin Resistance Frequencies among *Staphylococcus sp.* Coagulase Negative Isolates in Al-Basrah Governorate, Iraq

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Abstract

Background: Antibiotics called macrolide, lincosamide and streptogramin B (MLS_B) are being used to treat staphylococci infections. Multiple pathways that impart resistance to MLS_B antibiotics have been confirmed to cause clinical failure. The present work aimed to determine the frequency of constitutive and inducible clindamycin resistant among coagulase-negative staphylococci (CoNS) isolates of different clinical samples in Al-Basrah governorate, Iraq.

Methods: The 28 CoNS, traditional techniques and the Vitek®2 system were used to identify the isolates. The disk diffusion technique was used to detect methicillin resistance and antibiotic sensitivity patterns via cefoxitin, gentamicin, ciprofloxacin, amikacin, teicoplanin, linezolid, doxycycline and vancomycin disks. Erythromycin and clindamycin antibiotic disks was used to detect the inducible and constitutive clindamycin resistance as well as a D-test according to CLSI guidelines.

Results: Among 28 CoNS isolated, the *Staphylococcus aureus* 11(39.29%), *Staphylococcus epidermidis* 7(25 %), *Staphylococcus haemolyticus* 4(14.29%) and *Staphylococcus saprophyticus* 3 (10.71%) were predominant isolated species. Out of 28 CoNS isolates, 15(53.57%) were methicillin resistant coagulase-negative staphylococci (MRCoNS) isolates and 13(46.43%) were methicillin sensitive coagulase-negative staphylococci (MSCoNS) isolates. The 15(53.57%) isolates out of 28 CoNS, showed erythromycin resistance while 6(40%) isolates out of 15 CoNS, showed inducible macrolide-lincosamide-streptogramin B (iMLS_B) and 2(13.3%) of CoNS isolated showed constitutive macrolide-lincosamide-streptogramin B (cMLS_B).

Conclusions: In order to achieve the best result in choosing the suitable treatment and avoiding the loses the money and time, it is better to use the D-test for inducible clindamycin resistance in the daily routine work of antibiotic susceptibility testing in hospital and private clinical laboratories.

Keywords: Anti-Bacterial Agents, Clindamycin, *Staphylococcus*.

Introduction

Since the 1950s, coagulase-negative staphylococci (CoNS) have been recognised as an important cause of human infection (1-5). Antimicrobials macrolide-lincosamide-streptogramin B (MLS_B) family are commonly used to treat skin and soft tissue infections caused by CoNS (2), and also as a penicillin substitute in individuals who are allergic to penicillin (6).

Resistance to antibiotics in the MLS_B family by clinical isolates of *S. aureus* could be either constitutive (cMLS_B) or inducible (iMLS_B). Although rRNA methylase is only produced in the presence of an inducing agent, which can also be another antibiotic from MLS_B family, like erythromycin, or macrolide, and rRNA methylase is frequently created in the absence

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of an inducing agent in constitutive resistance (7-10).

Since erythromycin generates iMLS_B resistance, when using an erythromycin disc in relatively close proximity to a clindamycin disc (D-test) assists in capable of detecting this form of resistance in CoNS. Clindamycin treatment could fail if iMLS_B resistance isn't established (9-11). Therefore, current research used D-test to determine the frequency inducible clindamycin resistant among CoNS isolated isolates from different the clinical samples from Al-Basrah governorate, Iraq.

Materials and Methods

Ethical approval

The research has been approved by research ethics committee of college of science, department of biology, university of Basrah (No:2018/212).

Collection of specimens

Through September-2019 to December-2019, a total of 160 samples were collected from different clinical samples distributed to pus (n= 40), skin infections (n= 40), surgical wounds (n= 40), and nose swabs (n= 40).

Isolation and identification

The coagulase-negative staphylococci (CoNS) strains were identified according to conventional methods of Freney et al. (11). The first and the second steps including the confirmed identify were done by Vitek[®]2 system.

Antibiotic sensitivity test

Methicillin resistance was detected by using cefoxitin (30 µg; Mylan Teoranta Limited Company) disc according to method of CLSI-2018 (12).

Antibiotic sensitivity pattern

The gentamicin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), teicoplanin (30 µg), linezolid (30 µg), doxycycline (30 µg), and vancomycin (30 µg) were used to detect the sensitivity pattern according to CLSI-2015 (12).

Constitutive and inducible clindamycin resistance

Erythromycin (15 µg), antibiotics and (2 µg), and clindamycin was detected for inducible and constitutive resistance clindamycin according to guidelines of CLSI-2012 (13).

Results

Total of 160 sample was collected from different clinical samples, through September to December 2019. The 70 (43.75%) samples were positive for bacterial growth, in which 17(24.3%) from pus, 21(30%) from skin infections, 14(20%) from surgical wounds and 18(25.7%) from nose swabs. While the 90(56.25%) samples have been recorded as negative bacterial growth. Identification of bacterial growth by using biochemical tests and Vitek[®] 2 have been revealed different bacterial species. *Staphylococcus aureus*: 11(39.29%), *Staphylococcus epidermidis*: 7(25 %), *Staphylococcus haemolyticus*: 4(14.29%) and *Staphylococcus saprophyticus*: 3 (10.71%) were the most frequent bacterial species followed by *Staphylococcus xylosum* 1(3.57%), *Staphylococcus schleiferi* 1(3.57%), and *Staphylococcus warneri* 1(3.57%) (Fig. 1).

The out of 28 CoNS isolated, 15(53.57%) isolates consisted of 9(32.143%) *S. aureus*, 4(14.29%) *S. epidermidis*, and 2(7.143%) *S. haemolyticus* were only showed the methicillin resistance characterization when tested by cefoxitin (30 µg) according to CLSI guidelines (CLSI, 2012), while the another 13(46.43%) isolates including 2(7.143%) *S. aureus*, 3(10.71%) *S. epidermidis*, 2(7.143%) *S. haemolyticus*, 3(10.71%) *S. saprophyticus*, 1(3.57%) *S. xylosum*, 1(3.57%) *S. schleiferi*, and 1(3.57%) *S. warneri* were given methicillin sensitive characterization (Table 1).

Comparative evaluation result of the antibiotic susceptibility pattern showed that both MRCoNS and MSCoNS had the high percentage of sensitivity against all antibiotic that used in this study except the MRCoNS that were shown the high resistance against the Ciprofloxacin (Table 2).

From 15 isolates, the 6(40%) isolates were shown the inducible clindamycin resistance (iMLS_B) characteristic, while 2(13.3%) isolates

was given constitutive clindamycin resistance (cMLS_B) characteristic and 7(46.7%) isolates was shown MS phenotypes (Table 3).

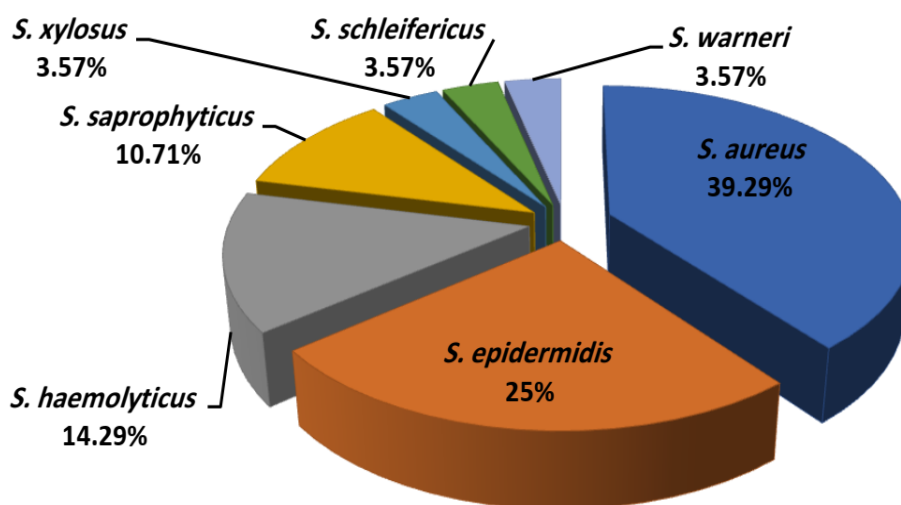


Fig. 1. The frequency of bacterial species isolated.

Table 1. Methicillin resistance distribution between *Staphylococcus* sp.

No.	Isolates	Methicillin expression		Isolates number and percentage
		MRCoNS	MSCoNS	
1.	<i>S. aureus</i>	(32.143%) ^a	2 (7.143%)	11 (39.29%)
2.	<i>S. epidermidis</i>	4 (14.29%)	3 (10.71%)	7 (25 %)
3.	<i>S. haemolyticus</i>	2 (7.143%)	2 (7.143%)	4 (14.29%)
4.	<i>S. saprophyticus</i>	•	3 (10.71%)	3 (10.71%)
5.	<i>S. xylosum</i>	•	1 (3.57%)	1 (3.57%)
6.	<i>S. schleifericus</i>	•	1 (3.57%)	1 (3.57%)
7.	<i>S. warneri</i>	•	1 (3.57%)	1 (3.57%)
Total		15 (53.57%)	13 (46.43%)	28

Table 2. *Staphylococcus* sp. susceptibility pattern of antibiotic comparison among MRCoNS and MSCoNS.

No.	Antibiotics tested	Methicillin expression			
		MRCoNS* N= 15		MSCoNS** N= 13	
		Resistant	Sensitive	Resistant	Sensitive
1.	Gentamicin	4 (26.7%)	11 (73.3%)	1 (7.7%)	12 (92.3%)
2.	Amikacin	2 (13.3%)	13 (86.7%)	0	13 (100%)
3.	Linezolid	1 (6.7%)	14 (93.3%)	0	13 (100%)
4.	Doxycycline	3 (20%)	12 (80%)	1 (7.7%)	12 (92.3%)
5.	Teicoplanin	1 (6.7%)	14 (93.3%)	0	13 (100%)
6.	Vancomycin	•	15 (100%)	0	13 (100%)
7.	Ciprofloxacin	12 (80%)	3 (20%)	2 (15.38%)	11 (84.62%)

Table 3. Methicillin resistant coagulase-negative staphylococci Sensitivity pattern for Erythromycin-Clindamycin with D-test.

No.	Isolates MRCoNS*	Pattern for Erythromycin-Clindamycin with D-test for MRCoNS resistant					
		ER(S) and CL(S)	iMLSB		MS phenotypic		cMLSB
			ER(R) and CL(S)	D-test	ER(R) and CL(S)	D-test	
1.	<i>S. aureus</i>	•	2 (13.3%)	+	5 (33.3%)	-	2 (13.3%)
2.	<i>S. epidermidis</i>	0	3 (20%)	+	1 (6.7%)	-	0
3.	<i>S. haemolyticus</i>	0	1 (6.7%)	+	1 (6.7%)	-	•
Total			6 (40%)		7 (46.7%)		2 (13.3%)
15							

Discussion

Antibiotic susceptibility testing clinical isolate is frequently required to effective antimicrobial treatment infected individuals. This is especially crucial in view of increasing resistance and the development of multidrug-resistant pathogens (15). The CONS strains, which are prevalent mucous and skin membrane pathogens, reported commonly as nosocomial infections. Antibiotic resistance, particularly the methicillin resistance, is challenge clinical treatment of these pathogens (16-18).

MRSA has become one of the most frequent nosocomial infections. It is critical to diagnose MRSA early and implement an antimicrobial therapy strategy (19, 20). In this study 53.57% methicillin resistance was discovered in of the isolates tested (Table 1). In 2008 the United States, MRSA was found in 55.7 % and 48.7 % of inpatients and outpatients, respectively (21). Shoja et al. (22) was reported the prevalence of 41%. Koppad et al. (23) found a prevalence of 54.9%, and also 62.4% in MRSA reported from Iran (17).

While in north India methicillin resistant was 38.8% (8). The varied MRSA frequency provided by different nations encourages targeted surveillance to collect local resistance data, which can lead to the most effective treatment (7, 20, 24). Resistance rates of CONS isolates to antibacterial in this study (shown in table 2) was lower than the result of study of Aghazadeh *et al.*, (17). However, the percentage of resistant was higher among

MRCONS isolates and this result was similar with the result of Khatoon and Jahan (8).

Erythromycin is the most commonly given antibiotic for staphylococcal infections, both mild and severe. Because of the rising prevalence of erythromycin resistance, the therapeutic options for staphylococcal infections are becoming increasingly restricted. Clindamycin is the medication of choice for treating MRSA infections (23) and also it is an excellent substitute for vancomycin. Its tolerability, affordability, excellent absorption, and ease of tissue penetration make it a necessary and exceptional good alternative for the treatment of patients (24). However, failure during therapy, which is mostly caused by inducible resistance phenotypes, is a significant concern in clindamycin treatment. A treatment choice cannot be made without appropriate antibiotic susceptibility testing, and that's where the D-test becomes vital and crucial (25).

In present study, out of 15 CONS isolates, 6(40%) showed resistance to erythromycin with D-test positive and inducible clindamycin resistance (iMLSB) was most predominant phenotype. While the MS phenotype and constitutive clindamycin resistance (cMLSB) were found to be 2(13.3%) and 7(46.7%), respectively (Table 3). Study of Khan et al. (26) was reported the inducible MLSB phenotype was shown to be more common in MRSA isolates. Koppad et al. (23) found that both MRSA and MRCNS isolates have a

significant frequency of constitutive and inducible MLS_B phenotypes. While Date et al. (25) reported that the inducible MLS_B phenotype in MRCON was less than the MS phenotype and constitutive phenotype ($cMLS_B$).

For the treatment of MRSA infections, there are only a few options, with clindamycin. As a result, genuine clindamycin sensitivity may be determined by running a simple D-test on all erythromycin-resistant *staphylococcus* species (27). Clindamycin therapy failure can be significantly reduced by conducting this easy test on a regular basis (28).

References

1. Bhakdi S, Trantum-Jensen J. Alpha-toxin of *Staphylococcus aureus*. *Microbiol rev.* 1991;55(4):733-51.
2. Otto M. *Staphylococcus* colonization of the skin and antimicrobial peptides. *Expert Rev Dermatol.* 2010;5(2):183-195.
3. Green BN, Johnson CD, Egan JT, Rosenthal M, Griffith EA, Evans MW. Methicillin-resistant *Staphylococcus aureus*: an overview for manual therapists. *J Chiropr Med.* 2012;11(1):64-76.
4. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nature Rev Microb.* 2019;17(4):203-218.
5. Abdullah-Al-Shoeb M, Huq S, Abul Kalam Azad M. Assessment of antibacterial efficacy of Lugol's iodine compared with commercial hand sanitizers of Bangladesh. *Journal of World's Poultry Research.* 2019;9(5):130-137.
6. Bora P, Datta P, Gupta V, Singhal L, Chander J. Characterization and antimicrobial susceptibility of coagulase-negative staphylococci isolated from clinical samples. *J Lab Physicians.* 2018;10(4):414-419.
7. Sasirekha B, Usha MS, Amruta JA, Ankit S, Brinda N, Divya R. Incidence of constitutive and inducible clindamycin resistance among hospital-associated *Staphylococcus aureus*. *3 Biotech.* 2014;4(1):85-89.
8. Khatoon R, Jahan N. Evaluation of Prevalence of Inducible Clindamycin Resistance among Coagulase Negative Staphylococci (CoNS)

The D test for inducible clindamycin resistance should be added to the daily routine work of antibiotic susceptibility testing in hospital and private clinical laboratories, for achieving the best result, the suitable, fast, and cost-effective treatment and avoid the lose of the money and time.

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Isolated from Various Clinical Samples in a Tertiary Care Hospital of North India. *International Journal of Current Microb Appl Sci,* 2018;7(2):513-522.

9. Chika E, Joseph NF, Chijioke E. Detection of constitutive and inducible-clindamycin-resistance in clinical isolates of *Staphylococcus aureus* from a Federal Teaching Hospital in Abakaliki, Nigeria. *Epidemiology of multidrug-resistant organisms in South-East Nigeria.* 2018;2(1):31-34.
10. Malek-Jafarian M, Hosseini F, Ahmadi A. Pattern of Infection and Antibiotic Activity among *Streptococcus agalactiae* Isolates from Adults in Mashhad, Iran. *Rep Biochem Mol Biol.* 2015;3(2):89-93.
11. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microb Rev.* 2014;27(4):870-926.
12. Frenay J, Kloos WE, Hajek V, Webster JA, Bes M, Brun Y, et al. Recommended minimal standards for description of new staphylococcal species. Subcommittee on the taxonomy of staphylococci and streptococci of the International Committee on Systematic Bacteriology. *Int J Syst Bacteriol.* 1999;49 Pt 2:489-502.
13. Institute, CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. 28th edn. Edited by P. Wayne. Wanyne, PA Clinical and Laboratory Standards Institute Antimicrobial, 2018.

14. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
15. Phukan C, Ahmed G, Sarma P. Inducible clindamycin resistance among *Staphylococcus aureus* isolates in a tertiary care hospital of Assam. Indian J Med Microbiol. 2015;33(3):456-8.
16. Piette A, Verschraegen GE. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134(1-2):45-54.
17. Aghazadeh M, Ghotaslou R, Rezaee MA, Moshafi MH, Hojabri Z, Saffari F. Determination of antimicrobial resistance profile and inducible clindamycin resistance of coagulase negative staphylococci in pediatric patients: the first report from Iran. World J Pediatr. 2015;11(3):250-4.
18. Tahmasebi S, Qasim MT, Krivenkova MV, Zekiy AO, Thangavelu L, Aravindhan S, et al. The effects of oxygen-ozone therapy on regulatory T-cell responses in multiple sclerosis patients. Cell Biol Int. 2021;45(7):1498-1509.
19. Pai V, Rao VI, Rao SP. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* [MRSA] isolates at a tertiary care hospital in Mangalore, South India. J Lab Physicians. 2010;2(2):82-4.
20. Seifi N, Kahani N, Askari E, Mahdipour S, Naderi NM. Inducible clindamycin resistance in *Staphylococcus aureus* isolates recovered from Mashhad, Iran. Iranian J Microb. 2012;4(2):82-86.
21. Pillar CMM. Prevalence of multidrug-resistant, methicillin-resistant *Staphylococcus aureus* in the United States: findings of the stratified analysis of the 2004 to 2005 LEADER Surveillance Programs. Diag Microbiol Infect Dis. 2008;60(2):221-4.
22. Shoja S, Nahaei MR, Nahaei M. Detection of inducible clindamycin resistance in *Staphylococcus aureus* and *Staphylococcus epidermidis* by using D-Test. Pharma Sci. 2009;15(1):1-8.
23. Koppad M, Parameshwar S, Halesh LH, Siddesh KC. Detection of inducible clindamycin resistance in *staphylococcus aureus* and CONS at tertiary care hospital. Indian Journal of Microbiology Research. 2015;2(4):192-197.
24. Debnath A, Ghosh R, Ghosh D. Debnath, A., Ghosh, R. and Ghosh, D. Detection of Inducible Clindamycin Resistance (iMLS B) among the Erythromycin Resistant CONS Isolates in a Rural Tertiary Care Hospital- Need of Time. International Journal of Health Sciences and Research (IJHSR). 2020;10:12-18.
25. Date K, Choudhary M, Thombare V. Inducible clindamycin resistance in clinical isolates of staphylococci in a rural hospital. Int J Biol Med Res. 2012;3(3):1922-5.
26. Khan F, Ali S, Sultan A, Rizvi M, Khatoon A, Shukla I, et al. A study of inducible clindamycin resistance in erythromycin resistant clinical isolates of *staphylococcus* species. Asian J Med Sci. 2015;6(6):48-52.
27. Shahmoradi M, Faridifar P, Shapouri R, Mousavi S F, Ezzedin M, Mirzaei B. Determining the Biofilm Forming Gene Profile of *Staphylococcus aureus* Clinical Isolates via Multiplex Colony PCR Method. Rep Biochem Mol Biol. 2019;7(2):181-188.
28. Shabgah AG, Qasim MT, Mostafavi SM, Zekiy AO, Ezzatifar F, Ahmadi M, et al. CXC chemokine ligand 16: a Swiss army knife chemokine in cancer. Expert Rev Mol Med. 2021;23:e4.