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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 achive 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* xylosus 1(3.57%), *Staphylococcus schleifericus* 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. xylosus*, 1(3.57%) *S. schleifericus*, 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).

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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).

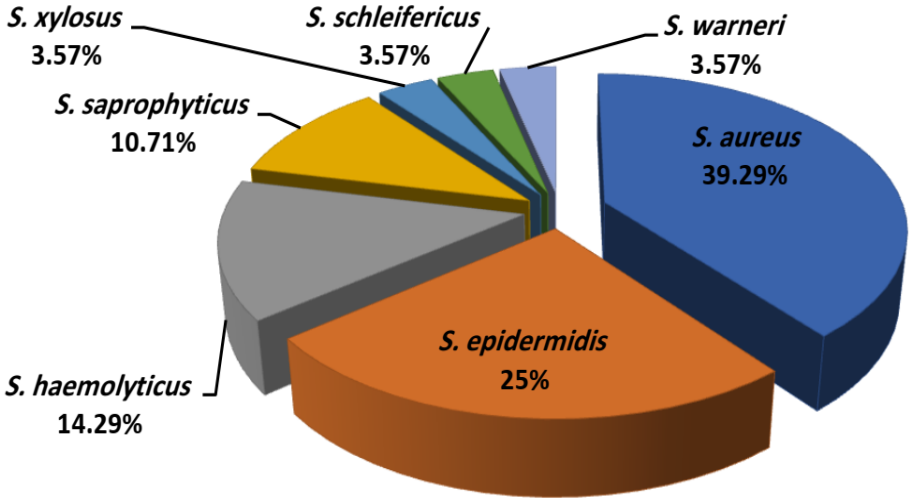

Fig. 1. The frequency of bacterial species isolated.

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

No.	Isolates	Methicillin expression		Isolates number and percentage
		MRCoNS	MSCoNS	
1.	*S. aureus*	(32.143%)٩	2 (7.143%)	11 (39.29%)
2.	*S. epidermidis*	4 (14.29%)	3 (10.71%)	7 (25 %)
3.	*S. haemolyticus*	2 (7.143%)	2 (7.143%)	4 (14.29%)
4.	*S. saprophyticus*	٠	3 (10.71%)	3 (10.71%)
5.	*S. xylosus*	٠	1 (3.57%)	1 (3.57%)
6.	*S. schleifericus*	٠	1 (3.57%)	1 (3.57%)
7.	*S. warneri*	٠	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	٠	15 (100%)	0	13 (100%)
7.	Ciprofloxacin	12 (80%)	3 (20%)	2 (15.38%)	11 (84.62%)

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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	ER(R) and CL(R)
1.	*S. aureus*	٠	2 (13.3%)	+	5 (33.3%)	-	2 (13.3%)
2.	*S. epidermidis*	0	3 (20%)	+	1 (6.7%)	-	0
3.	*S. haemolyticus*	0	1 (6.7%)	+	1 (6.7%)	-	٠
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 ir 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 ($iMLS_B$) was most predominant phenotype. While the MS phenotype and constitutive clindamycin resistance ($cMLS_B$) 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

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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).

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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