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Original Research Article

Inducible Clindamycin Resistance among Staphylococcal clinical isolates from Tripoli Central Hospital, Libya

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ABSTRACT:

BACKGROUND: The resistance to antimicrobial agents among Staphylococci is an increasing problem. This has led to a renewed interest in the usage of macrolide-lincosamide-streptogramin B (MLSB) antibiotics to treat staphylococcal infections. Clinical failure has been reported due to multiple mechanisms that confer resistance to clindamycin antibiotics. The present study was to investigate the inducible clindamycin resistance among isolates of methicillin resistant Staphylococci by the D-test method.

MATERIALS & METHODS: This study was conducted on 218 staphylococcal isolates obtained from different clinical specimens of outpatients and inpatients admitted to Tripoli Central Hospital (TCH), Libya. Methicillin resistance was detected by oxacillin, cefoxitin disc diffusion test (Kirby Bauer method) and confirmed by other biochemical tests. Detection of inducible clindamycin resistance was performed by D-test using erythromycin and clindamycin.

RESULTS: Eighty-six out of 218 staphylococcal isolates were resistant to erythromycin,**26** (11.9%) isolates were D-test positive indicating inducible ($iMLS_B$) phenotype, 24 (11%) isolates exhibited constitutive ($cMLS_B$) phenotype, while 36 (16.5%) showed true sensitivity to clindamycin indicating (MS) phenotype. The distribution of isolates showing $iMLS_B$ phenotype was 12 (19.4%) for methicillin-resistant *Staphylococcus aureus* (MRSA), 8 (17.0%) for methicillin-resistant coagulase-negative *Staphylococci* (MRCNS), 6 (6.4%) for methicillin-sensitive *Staphylococcus aureus* (MSSA) and 0 (0%) for methicillin-sensitive coagulase-negative *Staphylococci* (MSCNS).

CONCLUSION: Higher prevalence of iMLSB phenotype was mainly associated with methicillin-resistant than methicillin-sensitive isolates. We recommend that D-test should be performed to facilitate the appropriate treatment of patients infected with Staphylococci.

KEYWORDS: MRSA; MRCNS; clindamycin; erythromycin; inducible resistance; D-test.

INTRODUCTION

Coagulase-Positive *Staphylococci* (CPS) and coagulase-negative *Staphylococci* (CNS) are recognized as important pathogens that cause nosocomial and community acquired infections in every region of the world. The rising prevalence of methicillin resistance

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among *Staphylococci* is an escalating problem [1], which has renewed the attention for using other effective drugs to treat staphylococcal infections, such as the (MLS_B) antibiotics, which act through the common mechanism of protein synthesis inhibition, and are widely used to treat such infections [2]. Clindamycin (a lincosamide) is the agent preferred by clinicians due to its excellent pharmacokinetic properties [3]. The wide spread use of the MLS_B family of antimicrobials has led to the emergence of resistance to this group of antibiotics [4].

The macrolide antibiotic resistance in *Staphylococci* can be mediated by the macrolide streptogramin (*msr*) *A* gene (MS phenotype) which codes for an efflux mechanism that confer resistance to the macrolides and the type B streptogramin only, which has been more prevalent in CNS than in *S. aureus* or via the erythromycin ribosome methylase (*erm*) gene designated the MLS_B phenotype [4]. The expression of the MLS_B phenotype may be constitutive ($cMLS_B$) or inducible ($iMLS_B$) [5]. Patients infected with $iMLS_B$ strains of *Staphylococci* if treated with clindamycin can develop resistance during therapy resulting in treatment failure [6]. The MS and $iMLS_B$ phenotypes are indistinguishable by using standard susceptibility test methods. For the $iMLS_B$ strains, erythromycin will induce production of the methylase, which allows clindamycin resistance to be expressed [7]. This inducible clindamycin resistance can be detected with an erythromycin-clindamycin simple disk approximation test, commonly referred to as D-test as described by Fiebelkorn *et al*. [7].

Patients & Methods

This prospective study was conducted on (218) non-repeating isolates of *Staphylococci* obtained from various clinical specimens (pus swabs, drains, blood cultures, urine, sputum, vagina swabs, nasal swabs, ear swabs, ear swabs, throat swabs and urethral discharge) of outpatients visiting and inpatients admitted to Tripoli Central Hospital (TCH), Libya during the period from June 2013 to June 2014. The isolates were fully identified by standard conventional laboratory methods. MRSA and MRCNS isolates were initially identified using oxacillin (1 µg) and cefoxitin (30 µg) disks (Oxoid -UK). An inhibition zone of ≤ 10 mm around oxacillin disk indicates methicillin resistance. In regard to cefoxitin disk, an inhibition zone of ≤ 21 mm was considered as methicillin resistant in accordance to Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. In addition, the methicillin resistant isolates were subjected to chromogenic MRSA media (BioMerieux–France) and oxacillin screening media supplemented with 4% NaCl and oxacillin (6 µg/ml) (Becton Dickinson BDBBL).

For the detection of inducible clindamycin resistance, each isolate showed which was resistant to erythromycin was subjected to D-test by placing erythromycin (15µg) and clindamycin (2 µg) disc on Mueller-Hinton agar (BioMe´rieux, France) at adjacent positions, 15mm apart. Isolates resistant to erythromycin and having a clindamycin zone ≥ 21 mm with a flattened D-shaped zone in the area between the two discs were regarded as positive test for inducible resistance ($iMLS_B$ phenotype) [7,8]. Isolates exhibiting resistance to erythromycin but sensitive to clindamycin, giving circular zone of inhibition, were considered negative for D-test (MS phenotype), meanwhile, those staphylococcal isolates resistant to both erythromycin and clindamycin were regarded as constitutively resistant ($cMLS_B$ phenotypes). Isolates sensitive to both erythromycin and clindamycin were regarded as susceptible strains.

Staphylococcus aureus ATCC 25923 was used as the control strain.

Statistical analysis

Statistical analysis of the results were presented as frequencies and percentages using Microsoft Excel 2003 (version 11, Microsoft Corporation WA, USA).

Results

The majority of the isolates were obtained from pus swabs 174/218 (79.8%), followed by blood cultures 13 (6.0%), drain samples 9 (4.1%) and ear swabs 8 (3.7%). A total of 218 non-duplicate *Staphylococcus* species were isolated from different clinical specimens by the Microbiology Laboratory at TCH. and 156 (71.6%) were identified as CPS and 62(28.4%) were CNS. Within CPS isolates, 62(39.7%) were MRSA and 94 (60.3%) were MSSA. Among the coagulase-negative isolates, 47(75.8%) were MRCNS and 15(24.2%) were MSCNS.

A total of 86 (39.4%) staphylococcal isolates were resistant to erythromycin. The prevalence of (ER-S, CL-S) phenotype was 60.6% (132/218), followed by the (MS) phenotype 16.5% (36/218) and ($iMLS_B$) phenotype 11.9% (26/218), whereas the ($cMLS_B$) phenotype was seen in only 11.0% (24/218) of the isolates. The percentage of $iMLS_B$ resistance was higher among

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MRSA19.4 % (12/62) and MRCNS 17.0% (8/47) isolates as compared with MSSA 6.4% (6/94) and MSCNS 0% isolates. The susceptibility to erythromycin and clindamycin among all staphylococcal isolates is shown in Table (1).

Table 1: Susceptibility to erythromycin and clindamycin among all staphylococcal isolates.*

Phenotype	*MRSA n=62 (%)*	*MSSA n=94 (%)*	*MRCNS n=47 (%)*	*MSCNS n=15 (%)*	*Total n=218 (%)*
ER-S, CL-S	31 (50)	80 (85.1)	13 (27.7)	8 (53.3)	132 (60.6)
ER-R, CL-R (cMLS$_B$)	14 (22.6)	2 (2.1)	8 (17.0)	0	24 (11.0)
ER-R, CL-S (D$^+$) (iMLS$_B$)	12 (19.4)	6 (6.4)	8 (17.0)	0	26 (11.9)
ER-R, CL-S (D$^-$) (MS)	5 (8.1)	6 (6.4)	18 (38.3)	7 (46.7)	36 (16.5)

* MRSA=methicillin-resistant *S. aureus*, MRCNS=methicillin-resistant coagulase negative *Staphylococci*, MSSA=methicillin-susceptible *S. aureus*, MSCNS=methicillin susceptible coagulase negative *Staphylococci*, ER=erythromycin, CL=clindamycin, R=resistant, S=susceptible, cMLS$_B$=Constitutive MLS$_B$ phenotype, iMLS$_B$ = inducible MLS$_B$ phenotype, MS=MS phenotype, (D$^+$)=D-test positive, (D$^-$)=D-test negative.

DISCUSSION

Resistance to the majority of antibiotics used in the treatment of staphylococcal infections is an escalating problem [9]. The changing pattern in antibiotic susceptibility has led to a renewed interest in the use of clindamycin [1]. Clindamycin has been frequently used to treat skin and bone infections caused by staphylococcal species because of its low cost, good oral absorption and excellent tissue penetration making this drug an important option in outpatient therapy and change over after intravenous therapy. It is also used as an alternative in penicillin-allergic patients [7, 10]. Therapeutic failures caused by iMLS$_B$ resistant strains are now being commonly reported. Routine antimicrobial sensitivity testing can detect cMLS$_B$ phenotypes but iMLS$_B$ resistance is missed if erythromycin and clindamycin discs are placed at non-adjacent sites [7,11]. In our study we found that the prevalence of erythromycin–resistant staphylococcal isolates was 39.4% (86/218) which is slightly lower than that reported by previous local study (46%) [12] and a regional study(52.2%) [13].

In the present study, the prevalence of iMLS$_B$, cMLS$_B$ and MS resistance phenotype was 11.9%, 11.0% and 16.5% respectively. These findings are quite similar to those of Zorgani *et al.* [12] who found that in Tripoli, 27% of staphylococcal isolates were of the iMLS$_B$ phenotype whilst 3.2% and 15.1%exhibited the cMLS$_B$ and MS phenotypes respectively. Another recent study from Benghazi, reported that 4.5% of staphylococcal isolates had the iMLS$_B$ phenotype and 7.1 % were constitutively resistant and the MS phenotype constituted only 2.7% of the isolates [14]. Researchers from Egypt reported that the percentages of iMLS$_B$, cMLS$_B$ and MS resistance phenotypes were 7.7%, 6.6 % and 37.7 % respectively [13]. Such differences in the MLS$_B$-resistance pattern could be caused by differences in guidelines for drug usage in each country and is likely to vary by region. Various studies have shown the prevalence of the cMLS$_B$ phenotype to range from 11 to 27% and the MS$_B$ phenotype from 12 to 44% [15]. In the present study, infections caused by the MS phenotype isolates (16.5%), were treatable with clindamycin without fearing the emergence of resistance during therapy. But (11.9%) of patients infected with iMLS$_B$ strains if treated with clindamycin might develop resistance during therapy resulting in treatment failure.

A comparison of the prevalence rates of iMLS$_B$ isolates within the methicillin-susceptible staphylococci in different studies is displayed in Table 2.

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Our study shows that the prevalence rates of $iMLS_B$ among methicillin resistant strains were higher (19.4% for MRSA and 17.0% for MRCNS) than in methicillin sensitive isolates (6.4% in MSSA and 0% in MSCNS). This finding is concordant with those reported by most other studies [1,12,13,14,16,17,18] where the $iMLS_B$ was also found to be more common among the methicillin resistant staphylococcal isolates. It is clearly evident from these studies that the incidence of clindamycin resistance and the MLS_B phenotypes varies significantly between clinical isolates from different geographical regions [1,12,13,14,16,17,18] (Table 2).

Table 2: The percentage of inducible clindamycin resistance ($iMLS_B$) in staphylococci isolates from various studies.

Studies	MRSA %	MSSA %	MRCNS %	MSCNS %
Present study	19.4	6.4	17.0	0.0
Yilmaz G *et al.*, (1)	24.4	14.8	25.7	19.9
Zorgani A *et, al.*, (12)	66.2	0.0	-	-
Kilany A Amira (13)	5.1	0.0	-	-
Baiu H Saleh et. al., (14)	6.9	2.4	-	-
Pal N *et al.*, (16)	43.6	6.9	43.6	6.0
Baragundi M. *et al.*, (17)	24.4	12.0	16.4	3.2
Mojtaba M *et al.*, (18)	29.0	2.4	-	-

CONCLUSION

There is a high prevalence of inducible clindamycin resistance ($iMLS_B$) phenotype in methicillin-resistant compared with methicillin-sensitive isolates. The D-test is an easy, sensitive, and reliable means for detection of $iMLS_B$ strains in a clinical laboratory setting without specialized testing facilities. D-test reporting should continue to be done routinely for staphylococcal infections in order to reduce morbidity and mortality associated with inadvertent delay in administering the appropriate antibiotic treatment for these potentially serious maladies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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ملخص باللغة العربية

المقاومة المحرضة للكليندااميسين بين المكورات العنقودية المعزولة من المرضى في مستشفى طرابلس المركزي، ليبيا

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الملخص

نبذة مختصرة: مقاومة المضادة الحيوية للميكروبات بين المكورات العنقودية هي مشكلة متزايدة. وقد أدى ذلك زيادة الاهتمام باستخدام المضادات الحيوية مثل ماكروليد- لينكوساميد- ستربتوغرامين B لعلاج الالتهابات العنقودية. هناك تقارير عن فشل العلاج بالكليندااميسين لهذه الالتهابات بسبب عدة طرق من المقاومة.

أهداف الدراسة: الهدف من هذه الدراسة هو التحقيق في المقاومة المحرضة للكلندااميسين بين عزلات المكورات العنقودية المقاومة للميثيسيلين بواسطة طريقة الاختبار D.

الأساليب والمواد: أجريت هذه الدراسة على 218 عزلة من البكتريا العنقودية تم الحصول عليها من عينات سريرية مختلفة لمرضى في مستشفى طرابلس المركزي، ليبيا. تم الكشف عن مقاومة الميثيسيلين بطريقة نشر قرص أوكساسيلين وسيفوكسيتين وتم التأكيد على ذلك بعض الاختبارات الكيميائية الحيوية الأخرى. تم الكشف عن مقاومة الكلندااميسين المحرض من قبل إختبار D باستخدام الاريثروميسين والكليندااميسين

النتائج: كانت ستة وثمانين عزلة من أصل 218 عزلة للمكورات العنقودية مقاومة للإريثروميسين، وكانت 26 (11.9٪) عزلة موجبة الاختبار D حيث اعطت النمط الظاهري المحرض ($iMLS_B$)، و(24) (11%) عزلة أظهرت النمط التأسيسي ($cMLS_B$)، في حين أن 36 (16.5 ٪) التي أظهرت حساسية حقيقية للكليندامايسين تبين النمط الظاهري (MS). وكان توزيع العزلات التي لها النمط الظاهري المحرض (iMLSB) 12 (19.4٪) بالنسبة للمكورات العنقودية المقاومة للميثيسيلين موجبة التخثر و8 (17.0٪) للمكورات العنقودية المقاومة للميثيسيلين سالبة التخثر و6 (6.4٪) للمكورات العنقودية الذهبية الحساسة للميثيسيلين وصفر (0٪) للمكورات العنقودية الغير ذهبية والحساسة للميثيسيلين.

الاستنتاج: ارتفاع معدل انتشار النمط الظاهري المحرض ($iMLS_B$) والمرتبط أساسا مع العزلات المقاومة للميثيسيلين من العزلات الحساسة للميثيسيلين. ونوصي إجراء اختبار D لتسهيل العلاج المناسب للمرضى المصابين بالمكورات العنقودية.

الكلمات المفتاحية: MRCNS؛ كليندامايسين؛ الاريثروميسين؛ المقاومة المحرضة؛ اختبار D؛

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