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Macrolide and lincosamide resistance in staphylococcal clinical isolates in Nablus, Palestine

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Background/aim: Macrolide and lincosamide antibiotics are used for the treatment of staphylococcal infections, especially for penicillin-allergic patients. In the present study, we evaluate the prevalence of resistance to macrolide and lincosamide antibiotics among staphylococci isolates.

Materials and methods: A total of 200 staphylococcal clinical isolates were collected from January 2012 to April 2013. Minimal inhibitory concentrations of erythromycin and clindamycin were determined by agar dilution method. An erythromycin-clindamycin induction test was performed for isolates that were only resistant to erythromycin. Representative erythromycin-resistant isolates were examined for erythromycin resistance genes using PCR.

Results: Among staphylococci isolates, resistance frequencies of erythromycin and clindamycin were 65.5% and 20.5%, respectively. Erythromycin resistance was found to be mediated by putative efflux (50.4%) and target site modification (49.6%). Inducible target site modification resistance was detected in 19.1% of erythromycin-resistant isolates. Among the examined 36 staphylococci isolates, msr(A), erm(C), erm(A), and mef(A/E) genes were detected in 55.6%, 30.6%, 25%, and 0%, respectively.

Conclusion: Results of the current study indicate the presence of high rates of macrolide resistance and inducible phenotypes among staphylococcal isolates. It is also essential to keep in mind variations of resistance rates among various age groups and specimen types.

Key words: Macrolides, lincosamide, erm, msr(A), resistance, staphylococci

1. Introduction

Staphylococcus aureus is responsible for several diseases, such as toxic shock syndromes, bacteremia, skin infection, folliculitis, and boils. Coagulase-negative staphylococci (CONS) are an important cause of hospital-acquired infections, particularly nosocomial bacteremia (1).

The expanded therapeutic application of macrolide and lincosamide antibiotics has been accompanied by increased numbers of resistant strains among staphylococci (2,3). Two major mechanisms account for resistance to macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics in gram-positive bacteria (4). The target site modification mechanism of resistance is usually predominant. The mechanism depends on the methylase enzyme (encoded by *erm* genes), which causes ribosomal conformational changes rendering bacterial strains resistant to most macrolides, lincosamides, and streptogramin B compounds. Phenotypically, the pattern is known as macrolide-lincosamide-streptogramin B (MLS_B) resistance and its expression can be constitutive or inducible (4–7). In staphylococci, constitutive expression of MLS_B resistance can lead to cross-resistance to all macrolides, lincosamides, and streptogramin B ($cMLS_B$) (7). Strains with inducible resistance are resistant to inducer macrolides (possess 14- and 15-membered rings). By contrast, 16-membered ring macrolides, lincosamides (e.g., clindamycin), and streptogramin B compounds that are not inducers remain active. However, clindamycin therapy for inducible phenotypes can lead to clinical failure of treatment (7,8). The second mechanism is mediated by an efflux pump. Staphylococci appear to have a putative efflux system encoded by the *msr*(A) gene and possess specificity for macrolide and type B streptogramin molecules resulting in the MS-resistant phenotype (9,10). The streptococci efflux pump is specific for macrolides only and the resulting resistance pattern is referred to as phenotype M. In streptococci, an active efflux pump is encoded by *mef*(A) or *mef*(E) genes (11–13).

The aim of the current study was to determine the prevalence and phenotypes of resistance to macrolides and lincosamides among staphylococci clinical isolates in the Nablus district. In addition, PCR for representative isolates was carried out to detect macrolide resistance genes reported to occur in staphylococci and streptococci.

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2. Materials and methods

2.1. Design and settings of the study

The current study was a prospective research study conducted from January 2012 to April 2013 at An-Najah National University. The population of the Nablus district is approximately 364,000. Clinical isolates were collected from two governmental hospitals, Rafedia (200 beds) and Al-Watani (118 beds), and two private hospitals, Nablus (80 beds) and Al-Arabi (60 beds). In addition, isolates were also collected from two private medical laboratories (New Technology and Medicare). The study protocol was approved by the deans of An-Najah National University, the Palestinian Ministry of Health, and the directors of the participating clinical settings. All participating patients or their parents accepted inclusion of their bacterial cultures in the current study.

2.2. Bacterial isolates

Bacterial isolates were collected consecutively from different clinical settings in the Nablus district. Patients' clinical data were obtained from laboratory records. Isolates were given identification numbers and stored in 20% glycerol nutrient broth at –70 °C. Hospital-associated infections were defined as the occurrence of infection 48 h after hospital admission.

2.3. Identification of bacterial isolates

The identification of bacterial isolates was confirmed by several tests (1,14). Gram staining and catalase tests were performed for all isolates. Identification of staphylococcal bacteria was based on the coagulase test, mannitol salt agar test, aerobic production of acid from maltose, urease test, and susceptibility testing (bacitracin, novobiocin, and polymyxin B). All chemicals were obtained from Sigma-Aldrich (USA) and the used antibiotic disks were obtained from Oxoid (UK).

2.4. Determination of minimal inhibitory concentration (MIC)

MICs were determined by agar dilution method. Procedures including break points were carried out according to the Clinical Laboratory Standards Institute (CLSI) (1,15). *Staphylococcus aureus* ATCC 25923 was used as a control strain with susceptibility to both erythromycin and clindamycin antibiotics. Antibiotic powders were obtained from Sigma-Aldrich.

2.5. Detection of inducible MLS_B phenotype

Isolates resistant to erythromycin but susceptible to clindamycin were examined by double disk diffusion method (D-test) according to CLSI guidelines (16). Antibiotic disks were obtained from Oxoid.

2.6. Detection of methicillin resistance

Resistance to oxacillin antibiotics was used to indicate methicillin resistance among staphylococci isolates. Susceptibility to oxacillin was detected by disk diffusion method according to CLSI standards (15).

2.7. Detection of resistant genes

2.7.1. DNA extraction

Two to three bacterial colonies were suspended in a Tris acetate-EDTA (TAE) buffer for 1 h. Samples were then centrifuged at 1000 rpm for 5 min and pellets were resuspended and boiled in distilled water for 15 min. DNA was extracted by chloroform and DNA concentrations were measured using a spectrophotometer. Extracted DNA was stored at –20 °C until use.

2.7.2. Polymerase chain reaction (PCR)

The used primers and their sequences were *erm*(C): 5'-GCTAATATTGTTTAAATCGTCAATTCC-3', 5'-GGATCAGGAAAAGGACATTTTAC-3' (17); *erm*(B): 5'-GAAAAGGTACTCAACCAAATA-3', 5'-AGTAACGGTACTTAAATTGTTTAC-3' (18); *erm*(A): 5'-TCTAAAAAGCATGTAAAAGAA-3', 5'-CTTCGATAGTTTATTAATATTAGT-3' (2); *mef*(A/E) primer (targets *mef*(A) and *mef*(E) genes): 5'-AGTATCATTAATCACTAGTGC-3', 5'-TTCTTCTGGTACTAAAAGTGG-3' (18); *msr*(A) primer (targets *msr*(A) and *msr*(B) genes): 5'- GGCACAATAAGAGTGTTTAAAGG-3, 5'-AAGTTATATCATGAATAGATTGTCCTGTT-3' (18). PCR reagents were obtained from Sigma-Aldrich. The reaction mixture was modified according to Sutcliffe et al. (18). The PCR protocol was 5 min at 94 °C and 40 cycles of 1 min at 94 °C for the denaturation, 90 s at 45 °C for annealing, and 2 min at 72 °C for the extension step. The cycles were followed by a final extension step at 72 °C for 7 min. PCR products were detected by 1.5% agarose gels and stained with ethidium bromide.

2.8. Statistical analysis

Minitab 15.0 statistical analysis software was used. Chi-square or Fisher's exact tests were applied to compare the resistance frequencies among different groups. Independent t-tests were used to compare mean values among different age groups. For all analysis, $P < 0.01$ was considered statistically significant.

3. Results

3.1. Bacterial isolates

A total of 200 staphylococcal isolates were collected during the current study. The isolates comprised 187 *S. aureus* and 13 CONS (12 *S. epidermidis* and 1 *S. saprophyticus*). Isolates were recovered from different types of clinical specimens and only one positive culture per patient was included.

3.2. Resistance to antibiotics

Table 1 shows the resistance frequency among different bacterial species. A total of 131 (65.5%) staphylococci isolates were resistant to erythromycin. A much lower frequency of resistance to clindamycin (20.5%) was found

Table 1. Resistance to erythromycin and clindamycin in the studied bacterial isolates.

		Erythromycin			Clindamycin		
Bacteria species	No.*	Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)	Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
Staphylococci	200	131 (65.5)	6 (3)	63 (31.5)	41 (20.5)	6 (3)	153 (76.5)
S. aureus	187	121 (64.7)	6 (3.2)	60 (32.1)	39 (20.9)	5 (2.7)	143 (76.5)
CONS†	13	10 (76.9)	0 (0)	3 (23.1)	2 (15.4)	1 (7.7)	10 (76.9)
S. epidermidis	12	10 (83.3)	0 (0)	2 (16.7)	2 (16.7)	1 (8.3)	9 (75)
S. saprophyticus	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)

*No., number; †CONS, coagulase-negative staphylococci.

among these isolates. The frequency of erythromycin resistance among CONS was 76.9%, which was insignificantly higher than that among *S. aureus* (64.7%).

Frequency of erythromycin resistance (70.6%) among methicillin-resistant staphylococci isolates was insignificantly (P = 0.095) higher than that among methicillin- susceptible isolates (59.3%).

An erythromycin-clindamycin induction test was performed for isolates that were resistant to erythromycin but susceptible to clindamycin. MIC values for erythromycin and clindamycin, the erythromycin-clindamycin induction test, and detection of resistance genes by PCR in representative isolates were combined to predict the most probable mechanism of resistance. Erythromycin resistance of staphylococci isolates appeared to be mediated by a putative efflux mechanism (MS phenotype, 50.4%) and target site modification (MLS_B phenotypes, 49.6%). Staphylococci isolates with the target modification mode of resistance expressed the MLS_B phenotype constitutively and inducibly in 61.5% and 38.5% of the isolates, respectively. Thus, a considerable proportion of erythromycin-resistant isolates (19.1%) exhibited inducible MLS_B.

On the basis of resistance phenotypes and species of bacteria, 36 erythromycin-resistant representative isolates were examined for the presence of five resistance genes. Distribution of resistance genes among the different studied bacterial species and the predicted resistant phenotypes are shown in Table 2. Among the representative 36 staphylococcal isolates analyzed by PCR, the *msr*(A) gene was detected in 20 (55.6%), *erm*(C) in 11 (30.6%), and *erm*(A) in 9 (25%). The *mef* gene was not detected in any of the examined staphylococci isolates; however, it was detected in *Streptococcus agalactiae*, which was used as a positive control.

High percentages of erythromycin resistance were found among staphylococci isolates obtained from different clinical settings (Table 3). Staphylococci isolates from the Al-Watani hospital showed the highest resistance rates to erythromycin (85.7%) and clindamycin (85.7%). These rates were significantly higher than those found among isolates collected from the Rafedia hospital (P = 0.000).

With respect to erythromycin resistance, staphylococcal strains isolated from the gynecology department had the highest frequency (100%) compared to isolates from

Table 2. Macrolide resistance genes found in examined staphylococci isolates.

Type of bacteria and resistance phenotype	Examined isolates	Detected gene (%)				
		erm(A)*	*erm*(B)*	*erm*(C)*	*msr*(A)*	*mef*(A/E)*
Staphylococci	36	9 (25)	0 (0)	11 (30.6)	20 (55.6)	0 (0)
MLS_B constitutive	7	0 (0)	0 (0)	7 (100)	1 (14.3)	0 (0)
MLS_B inducible	11	8 (72.7)	0 (0)	4 (36.4)	1 (9.1)	0 (0)
MS	18	1 (5.55)	0 (0)	0 (0)	18 (100)	0 (0)

**erm*, erythromycin ribosome methylase; *msr*, macrolide and streptogramin B resistant; *mef*, macrolide efflux; *mef*(A/E), *mef*(A), or/and *mef*(E) gene(s) C, constitutive; I, inducible. CONS, coagulase-negative staphylococci.

Table 3. Clinical data of erythromycin and/or clindamycin-resistant staphylococci isolates

Variable	No. of isolates*	E. R* (%)	DA. R* (%)
Source			
Rafidia H*	140	90 (64.3)	22 (15.7)
New Technology Lab*	9	5 (55.6)	2 (22.2)
Nablus Specialty H*	21	12 (57.1)	6 (28.6)
Al-Arabi Specialty H*	18	14 (77.8)	3 (16.7)
Al-Watani H*	7	6 (85.7)	6 (85.7)
Medicare Lab*	5	4 (80)	2 (40)
Department			
Outpatients	56	41 (73.2)	14 (25)
Inpatients	144	90 (62.5)	27 (18.8)
General surgery	32	12 (37.5)	1 (3.1)
Emergency	17	9 (52.9)	0 (0)
Pediatrics	16	11 (68.8)	4 (25)
Burns	16	10 (62.5)	3 (18.8)
Neonates	14	12 (85.7)	5 (35.7)
Urology	14	8 (57.1)	3 (21.4)
ICU*	9	6 (66.7)	4 (44.4)
Internal medicine	13	10 (76.9)	4 (30.8)
Orthopedics	7	6 (85.7)	1 (14.3)
Gynecology	6	6 (100)	2 (33.3)
Specimen			
Wound swab	126	5 (59.5)	19 (15.1)
Urine	23	18 (78.3)	7 (30.4)
Blood	8	8 (100)	2 (25)
Sputum	6	4 (66.7)	4 (66.7)
Nasal swab	8	8 (100)	2 (25)
Various specimens (1–5)†	29	0-1 (0–100)	0–1 (0–100)
Sex			
Male	113	78 (69.9)	26 (26.5)
Female	87	53 (60.9)	15 (18.4)
Total	200	31 (65.5)	41 (20.5)

Abbreviations: E. R, erythromycin-resistant; DA. R, clindamycin-resistant; H, hospital; Lab, laboratory; ICU; intensive care unit.
† Specimens included those from skin, burn swab, tissue, chest swab, cerebrospinal fluid, pus, drainage, breast discharge, fluid, throat swab, vaginal swab, umbilical swab, ear swab, semen, or central venous catheter.

other departments, as well as outpatients' isolates (Table 3). Frequency differences were significant in comparison with those isolates obtained from outpatients and patients of general surgery, emergency, pediatrics, and burns departments (P = 0.000). On the other hand, the highest frequency of clindamycin resistance was found in intensive care units (44.4%), and it was also significantly higher than that of emergency departments (P = 0.001).

With respect to specimen types from which the bacteria were recovered, resistance to erythromycin was observed in all staphylococci isolates obtained from blood and nasal swabs (Table 3). This rate was significantly higher than

that found among wound swabs (P = 0.000). Resistance to clindamycin was highest in staphylococci bacteria isolated from sputum (66.7%).

As shown in Table 4, staphylococci isolates' resistance to erythromycin was highest among age group 0–2 years (74.5%) and age group >65 years (75%). In a similar manner, clindamycin resistance among staphylococci was highest in isolates collected from patients >65 years (50%), and this was significantly higher than that among the age group of 3–14 years (P = 0.007).

Evidence of nosocomial infection was found in 33 cases (32 *S. aureus* and 1 *S. epidermidis*). Among the 33 cases, 22 (21 *S. aureus* and 1 *S. epidermidis*) were erythromycin-resistant and 13 (12 *S. aureus* and 1 *S. epidermidis*) were erythromycin- and clindamycin-resistant. Findings on resistant phenotypes, antibiotic resistance profiles, and sources of isolates for several samples (in one hospital: pediatric, 2; urology, 2; and burns department, 3) indicated the relatedness of isolates and their role in nosocomial infections. To confirm this assumption, further molecular typing of these isolates is required.

Table 4. Distribution of erythromycin and clindamycin resistant staphylococcal isolates among different age groups

Age group	Total	No.*	E. R* (%)	DA. R* (%)
0-2 years	55	51	38 (74.5)	13 (25.5)
3-14 years	32	29	15 (51.7)	1 (3.5)
15-39 years	56	36	22 (61.1)	3 (8.3)
40-65 years	41	31	18 (58.1)	8 (25.8)
>65 years	14	12	9 (75)	6 (50)
Unknown	54	41	29 (70.7)	10 (24.4)

* No., number; E. R, erythromycin-resistant; DA. R, clindamycin-resistant.

4. Discussion

In the current study, a high frequency of erythromycin resistance among staphylococci isolates (65.5%) was found. Resistance to erythromycin was more frequent in CONS (76.9%) compared to that of *S. aureus* (64.7%). A study from Turkey reported a high resistance rate to erythromycin (59.2%) among staphylococci isolates collected from 2003 to 2005 (19). That study also reported a high resistance rate to erythromycin in CONS (69.8%) compared to *S. aureus* isolates (49.6%). Such a finding is in agreement with our results. This may be explained by the frequent presence of CONS as normal flora in patients before causing infection, a situation that allows longer exposure periods to antibiotics and consequently better conditions for natural selection of resistance.

In the present study, the erythromycin resistance rate (70.6%) among methicillin-resistant staphylococci isolates was insignificantly higher than that among methicillin-susceptible isolates (59.3%). The higher erythromycin resistance rate among methicillin-resistant staphylococci was linked to the presence of erythromycin-resistant genes conserved in *mec* DNA (20).

The prevalence of clindamycin resistance among staphylococci in our study (20.5%) was lower than that of erythromycin (65.5%). This can be attributed mainly to the frequent existence of the efflux mode of resistance (MS phenotype) among staphylococci in our region, as well as the induction capacity of erythromycin and not clindamycin for methylase enzyme production among inducible MLS_B phenotypes. A higher rate of clindamycin resistance among staphylococci (46.97%) was reported in India (21).

In the current study, resistance of staphylococci to erythromycin appeared to have been mediated by both efflux (MS phenotype, 50.4%) and target site modification (MLS_B phenotypes, 49.6%) mechanisms. Both inducible and constitutive MLS_B phenotypes, as well as MS phenotypes, were reported to occur, but with slightly different frequencies (21). Among the erythromycin-resistant staphylococcal isolates of the present study, 30.5% expressed the MLS_B phenotype constitutively and 19.1% inducibly. Thus, a considerable proportion of erythromycin-resistant isolates exhibited an inducible MLS_B phenotype. These isolates will appear susceptible to clindamycin in the disk diffusion method and will be at a high risk of conversion from inducible to constitutive MLS_B phenotype in vivo. As a result of conversion, one should expect clindamycin medication failure. Thus, the erythromycin-clindamycin induction test is essential to differentiate between strains carrying *erm* genes and fully susceptible clindamycin strains (22).

Variations in erythromycin and clindamycin resistance frequencies as well as resistant phenotypes in different parts of the world are expected to occur due to time factors, compliance with the use of antibiotics, the predominant species among studied genera, and outbreaks of resistant strains in different clinical settings.

In the current study, out of 36 examined staphylococci isolates, 9 (25%) possessed *erm*(A), 11 (30.6%) *erm*(C), and 20 (55.6%) *msr*(A). The *erm*(B) and *mef*(A/E) genes were not detected. A low prevalence of *erm*(B) among staphylococci was recorded in an earlier study (23). In addition, the presence of *mef* gene appeared to be limited to streptococci.

Variations in antibiotic resistance frequency among different clinical settings seemed to be influenced by hospital ward types. This was evident from the finding of a higher percentage of erythromycin and clindamycin

resistance among staphylococci isolates of Al-Watani Hospital, which is specialized mainly for internal medicine, where patients might be suffering from severe infections, compared to Rafedia Hospital, which is mainly a surgical hospital and admits patients most likely enrolled for surgery. Furthermore, erythromycin resistance among staphylococci isolates recovered from gynecology wards was significantly higher than that of other wards. Most of the women admitted to this ward were pregnant and/or admitted for delivery. Such cases are more sensitive to bacterial infection due to modulated immunity (24,25), resulting in prolonged bacterial infection periods.

All staphylococci bacteria isolated from blood and nasal swabs were resistant to erythromycin. Variations in resistance rates were significant when compared with wound swabs (P = 0.000). Many cases of bacteremia and septicemia are complications after primary infection in sites other than blood circulation (26). Usually a patient receives treatment before these complications. Therefore, the bacterial strains reaching the blood are expected to be resistant to the antibiotics used for the treatment of primary infections. The inside of the nasal cavity and the respiratory tract are known for their poor blood circulation. This is expected to decrease exposure of bacteria to the immune system of the host and decrease the exposure dose of antibiotics to bacteria. Under these conditions, bacteria will have the opportunity to develop antibiotic resistance. This might explain the high resistance rate to erythromycin among staphylococci strains isolated from nasal specimens. A high prevalence of multidrug resistance (nonsusceptibility to ≥4 antimicrobial classes) in MRSA nasal isolates was also reported by Davis et al. (27).

With respect to age groups, erythromycin resistance showed the highest rate among staphylococci isolates recovered from patients of 0–2 years and >65 years. In addition, the clindamycin resistance rate among patients of >65 years was significantly higher than in other groups. This could be due to the capacity of the immune system in these age groups. The current study shows clear variations, one of which was statistically significant, in resistance distribution among different age groups. Results of previous studies support our findings. For example, the findings of very high resistance rates to erythromycin among staphylococci isolated from neonates (90% of *S. epidermidis* and 100% of *S. haemolyticus* were resistant) are in agreement with our findings regarding the age group of 0–2 years (28). On the other hand, the findings of Adam et al. (29) on resistance of *S. aureus* and other pathogens to antibiotics (methicillin, clindamycin, and clarithromycin) are consistent with our findings among the age group of >65 years.

In conclusion, the results of the current study clearly indicate the presence of high macrolide-resistance rates among bacterial isolates collected from various clinical settings. In addition, a considerable proportion of macrolide resistance was due to inducible phenotypes, and thus it seems essential to carry out the induction test before any decision for clindamycin prescription. It is also essential to keep in mind variations of resistance rates among various age groups, specimen types, and pregnant women in particular.

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