Inducible Clindamycin Resistance in Beta-Hemolytic Streptococci and *Streptococcus pneumoniae*

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ABSTRACT: **Background:** Resistance to macrolides in beta-hemolytic streptococci and *Streptococcus pneumoniae* arises primarily due to Erm(B) or Mef(A). Erm(B) typically confers high level resistance to macrolides, lincosamides and streptogramin B (MLS_B phenotype), whereas Mef(A) confers low level resistance to macrolides only (M phenotype).

Objectives: To investigate the incidence of macrolide resistance mechanisms in isolates of beta-hemolytic streptococci and pneumococci in Israel, with particular emphasis on inducible MLS_B phenotype.

Methods: We collected 316 clinical isolates of streptococci during May–August 2010. Erythromycin resistance mechanism was determined by the erythromycin-clindamycin double disk diffusion method.

Results: Erythromycin and clindamycin resistance rates were 19.4% and 13.4% for *S. pneumoniae*, 4.7% and 1.6% for Group A Streptococcus (GAS), 17% and 17% for Group B Streptococcus (GBS), and 38.8% and 27.8% for Group G Streptococcus (GGS) respectively. The most common resistance mechanism for all streptococci was constitutive MLS_B ($cMLS_B$). Inducible MLS_B ($iMLS_B$) mechanism was found in 3% of all strains and represented 25% of resistance mechanisms.

Conclusions: The prevalence of macrolide resistance and the distribution of resistance mechanisms differ among β-hemolytic streptococci and *S. pneumoniae*, with GBS, GGS and *S. pneumoniae* showing the highest resistance rate. Macrolide or lincosamide cannot be empirically used for severe streptococcal infections before strains are proved to be susceptible. Continuous surveillance of erythromycin and clindamycin resistance patterns among streptococci is needed.

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KEY WORDS: clindamycin, erythromycin, beta-hemolytic Streptococcus, *Streptococcus pneumoniae*, $iMLS_B$

Penicillin has been the treatment of choice for various streptococcal infections, whereas erythromycin and clindamycin are usually recommended as alternative antibiotics for both beta-hemolytic streptococci and *Streptococcus pneumoniae* infections. The first erythromycin-resistant strain of Streptococcus was described in 1959 in Britain [1]. Since then, an increase in erythromycin and clindamycin resistance among beta-hemolytic streptococci and pneumococci has been observed worldwide, but with different prevalence rates reported among countries [2]. The most common mechanisms by which streptococci develop resistance to macrolides is by ribosomal target site modification that results from dimethylation of an adenine residue in the 23S rRNA, encoded by *Erm* genes, and efflux of the drug outside the organisms by macrolide efflux pump, encoded by *Mef* genes. Target site modification confers inducible (iMLSB) or constitutive (cMLSB) resistance to all antimicrobials in the macrolide-lincosamide-streptogramin B group.

The rates of erythromycin resistance among beta-hemolytic streptococci, mainly group A Streptococcus (*Streptococcus pyogenes*) [3], group B Streptococcus (*Streptococcus agalactiae*) [4] and *Streptococcus pneumoniae* [2], have been constantly increasing but vary among different regions in the world.

In Israel, erythromycin resistance varies among different strains. Resistance of GAS isolates to erythromycin (2%) and clindamycin (1%) remains low [5,6], while resistance of *S. pneumoniae* to erythromycin and clindamycin is increasing, reaching 30% and 22% respectively in some populations [7]. Data regarding macrolide resistance in other streptococci, and data regarding lincosamides resistance for all streptococci, are limited.

The aim of this study was to investigate the macrolide and lincosamide resistance patterns in clinical isolates of beta-hemolytic streptococci and *S. pneumoniae*.

MATERIALS AND METHODS

Isolates of beta-hemolytic streptococci and *Streptococcus pneumoniae* isolated from various body sites in the clinical microbiology laboratory of Shaare Zedek Medical Center during the period 1 May to 31 August 2010 were collected. Strains were serogrouped with a commercial latex agglutination kit (PathoDX Strep Grouping, Remel Inc., USA). Determination of macrolide-lincosamide-streptogramin B (MLS_B) phenotype was determined by a disk diffusion method in Mueller-Hinton medium supplemented with 5% defibrinated horse blood

GAS = group A Streptococcus

Table 1. Distribution of clinical isolates of β-hemolytic streptococci and pneumococci

Sample	GAS	GBS	GCS	GFS	GGS	*S. pneumoniae*	Total
Throat	92	1	1	0	0	0	94
Vagina	4	34	1	1	1	1	42
Blood	11	8	1	0	7	13	40
Wounds and abscesses	11	8	4	1	7	1	32
Sputum+pleural effusion*	0	0	0	0	1	27	28
Ears	7	1	0	0	0	20	28
Urine	0	28	0	0	0	0	28
Placenta	0	5	0	1	0	1	7
Abdominal**	0	1	0	2	2	0	5
Eyes	0	0	0	0	0	4	4
Sperm	0	2	0	0	0	0	2
Joints	2	0	0	0	0	0	2
Total	127	88	7	5	18	67	312

* Including sputum taken by broncho-alveolar lavage

** Including peritoneal swabs and intra-abdominal abscesses

GAS = group A Streptococcus, GBS = group B Streptococcus, GCS = group C Streptococcus, GFS = group F Streptococcus, GGS = group G Streptococcus

Table 2. Erythromycin and clindamycin resistance phenotypes among clinical isolates of β-hemolytic streptococci and pneumococci

	Total (n)	Erythro R n (%)	$cMLS_B$ n (% of total)	$iMLS_B$ n (% of total)	M Phenotype n (% of total)
Group A	127	6 (4.7%)	2 (1.6%)	0	4 (3.1%)
Group B	88	15 (17%)	8 (9.1%)	7 (7.9%)	0
Group C	7	0	0	0	0
Group F	5	0	0	0	0
Group G	18	7 (38.9%)	3 (16.7%)	2 (11.1%)	2 (11.1%)
S. pneumoniae	67	13 (19.4%)	8 (11.9%)	1 (1.5%)	4 (6%)
Total	312	41 (13.1%)	21 (6.7%)	10 (3.2%)	10 (3.2%)

(Hylabs, Rehovot, Israel) at 37.8ºC in 5% CO_2 [8]. Clindamycin disks (Becton Dickinson, USA) and erythromycin disks were placed at an edge-to-edge distance of 20 mm, and the induction of clindamycin resistance was assessed. The constitutive MLS_B phenotype is associated with high resistance to erythromycin and clindamycin. The inducible MLS_B phenotype is associated with resistance to erythromycin and susceptibility to clindamycin, with antagonism between the erythromycin and clindamycin disks (D shape). The M phenotype (efflux) is determined by resistance to erythromycin, susceptibility to clindamycin, and no antagonism between the two disks.

RESULTS

During the period May–August 2010 a total of 312 consecutive patient-specific clinical isolates of streptococci were collected and distributed as follows: 127 GAS, 88 GBS, 7 group C Streptococcus, 5 group F Streptococcus, 18 group G Streptococcus and 67 strains of *S. pneumoniae.* The distribution of clinical samples according to streptococcal serotype is shown in Table 1.

Altogether, there were 41 erythromycin-resistant and 31 clindamycin-resistant isolates. Erythromycin resistance was found in 13 pneumococcal isolates (19.4%), 6 group A streptococci (4.7%), 15 group B streptococci (17%) and 7 group G streptococci (38.9%). All group C and group F isolates were fully sensitive to both erythromycin and clindamycin. Clindamycin resistance was found in 9 pneumococcal isolates (13.4%), 2 group A streptococci (1.6%), 15 group B streptococci (17%) and 5 group G streptococci (27.8%). There were no strains with resistance to clindamycin and sensitivity to erythromycin. For pneumococcal isolates, no invasive strain (blood, cerebrospinal fluid, pleural effusion) was resistant to macrolides, while 19.4% of non-invasive pneumococcal strains were resistant to erythromycin ($P = 0.02$). Susceptibility phenotypes for all streptococcal isolates are shown in Table 2.

The most common resistance mechanism for all streptococci was $cMLS_B$, which was found in 6.7% of the isolates and represented 51% of resistance mechanisms. $iMLS_B$ and M phenotypes were each found in 3.2% of the GAS strains and represented 25% of resistance mechanisms. Macrolide-only resistance was found in 10 isolates, which denotes Mef A phenotype. It is possible that we did not recognize isolates with both MLS_B and Mef A phenotypes.

DISCUSSION

Resistance of various streptococci to macrolides has been constantly rising. In this study, we found a high rate of erythromycin and clindamycin resistance rates in pneumococci, GBS and GGS, with a lower resistance rate in GAS. Current data on macrolide and clindamycin resistance patterns for streptococci are limited.

Resistance of *Streptococcus pyogenes* to macrolides varies between different locations, ranging from 2% to 19% [3,9,10]. The relative proportion of $iMLS_B$ phenotype also differs between various places and ranges from 2% to 51% [11,12].

Streptococcus agalactiae (GBS) is frequently carried in the normal vaginal flora. It is an important cause of neonatal infection but also causes significant morbidity in adults. GBS resistance rates to erythromycin and clindamycin differ among different regions of the world, being relatively low in northern Europe [13], high in southern Europe [14], and highest in the Far East [15,16]. We found 17% erythromycin resistance in our population, with 8% having $iMLS_B$ phenotype. This finding has immediate clinical implica-

GBS = group B Streptococcus
GGS = group G Streptococcus

tions regarding prevention of perinatal GBS disease. Recent Center of Disease Control guidelines specifically require that the D-test be performed in all isolates in order to choose the optimal prophylactic antibiotic regimen. For clindamycin-resistant isolates or those with unknown clindamycin susceptibility, vancomycin is recommended [17]. Our findings, showing a relatively high rate of inducible clindamycin resistance among GBS isolates, support adoption of these recommendations.

Resistance to macrolides among *Streptococcus pneumoniae*, a common cause of community-acquired pneumonia and otitis media, has been increasing [18]. A large U.S. study that examined the rate of macrolide resistance among middle ear isolates of *S. pneumoniae* found that 37% of the strains were non-susceptible to erythromycin. Erythromycin resistance increased from 15% in 1994–95 to 56% in 1999–2000. Seventy-five percent of the strains remained susceptible to clindamycin [19]. Inducible clindamycin resistance has not been frequently evaluated for erythromycin-resistant pneumococci. A few studies did not find iMLSB phenotype in *S. pneumoniae*, even in places with a high rate of erythromycin resistance [20], while others found a lower rate of macrolide resistance but 2%–38% of resistant strains with iMLSB phenotype [17,19,21,22].

In our study, GGS, GBS and *S. pneumoniae* showed the highest resistance rates to both erythromycin and clindamycin. The high rate of resistance in GGS strains is in agreement with a previous study [23], but the reason for this finding is yet to be determined.

The incidence of macrolide resistance in streptococci has risen sharply in various regions of the world and may be due to the increased use of long-acting macrolides. This might also be the reason for the increase in resistance rates of streptococci to macrolides in Israel in recent years [24], and we should thus expect an increase in clindamycin resistance rates as well.

CONCLUSIONS

The iMLSB phenotype accounted for 25% of the erythromycin-resistant streptococci in this study. We therefore conclude that it is important to perform double disk-diffusion tests according to recommendations of the Clinical and Laboratory Standards Institute [25] to verify clindamycin resistance in a routine clinical diagnostic laboratory for all body fluid samples. Macrolides or clindamycin cannot be empirically used for severe streptococcal infections before strains are proven to be susceptible. Continuous surveillance of erythromycin and clindamycin resistance patterns among streptococci is needed to provide guidance for empiric therapy in cases of suspected streptococcal infections where β-lactams cannot be used.

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Capsule

MR1 presents microbial vitamin B metabolites to MAIT cells

Antigen-presenting molecules, encoded by the major histocompatibility complex (MHC) and CD1 family, bind peptide- and lipid-based antigens, respectively, for recognition by T cells. Mucosal-associated invariant T (MAIT) cells are an abundant population of innate-like T cells in humans that are activated by an antigen(s) bound to the MHC class I-like molecule MR1. Although the identity of MR1-restricted antigen(s) is unknown, it is present in numerous bacteria and yeast. Kjer-Nielsen and co-workers show that the structure and chemistry within the antigen-binding cleft of MR1 is distinct from the MHC and CD1 families. MR1 is ideally suited to bind ligands originating from vitamin metabolites. The structure of MR1 in complex with 6-formyl pterin, a folic acid (vitamin B9) metabolite, shows the pterin ring sequestered within MR1. Furthermore, the authors characterize related MR1-restricted vitamin derivatives, originating from the bacterial riboflavin (vitamin B2) biosynthetic pathway, which specifically and potently activate MAIT cells. Accordingly, they show that metabolites of vitamin B represent a class of antigen that are presented by MR1 for MAIT-cell immunosurveillance. As many vitamin biosynthetic pathways are unique to bacteria and yeast, these data suggest that MAIT cells use these metabolites to detect microbial infection.

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Capsule

Phosphorylation of NLRC4 is critical for inflammasome activation

NLRC4 is a cytosolic member of the NOD-like receptor family that is expressed in innate immune cells. It senses indirectly bacterial flagellin and type III secretion systems, and responds by assembling an inflammasome complex that promotes caspase-1 activation and pyroptosis. Qu et al. use knock-in mice expressing NLRC4 with a carboxy-terminal 3×Flag tag to identify phosphorylation of NLRC4 on a single, evolutionarily conserved residue, Ser 533, following infection of macrophages with *Salmonella enterica* serovar Typhimurium (also known as *Salmonella typhimurium*). Western blotting with a NLRC4 phospho-Ser 533 antibody confirmed that this post-translational modification occurs only in the presence of stimuli known to engage NLRC4 and not the related protein NLRP3 or AIM2. Nlrc4−/− macrophages reconstituted with NLRC4 mutant S533A, unlike those reconstituted with wild-type NLRC4, did not activate caspase-1 and pyroptosis in response to *S. typhimurium*, indicating that S533 phosphorylation is critical for NLRC4 inflammasome function. Conversely, phosphomimetic NLRC4 S533D caused rapid macrophage pyroptosis without infection. Biochemical purification of the NLRC4-phosphorylating activity and a screen of kinase inhibitors identified PRKCD (PKCδ) as a candidate NLRC4 kinase. Recombinant PKCδ phosphorylated NLRC4 S533 in vitro, immunodepletion of PKCδ from macrophage lysates blocked NLRC4 S533 phosphorylation in vitro, and Prkcd−/− macrophages exhibited greatly attenuated caspase-1 activation and IL-1β secretion specifically in response to *S. typhimurium*. Phosphorylation-defective NLRC4 S533A failed to recruit procaspase-1 and did not assemble inflammasome specks during *S. typhimurium* infection, so phosphorylation of NLRC4 S533 probably drives conformational changes necessary for NLRC4 inflammasome activity and host innate immunity.

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Targeted Therapy with Low Doses of ^{131}I-MIBG is Effective for Disease Palliation in Highly Refractory Neuroblastoma

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ABSTRACT: **Background:** Palliative treatment of refractory neuroblastoma remains a significant clinical problem.

Objectives: To retrospectively determine the clinical response to ^{131}I-MIBG therapy at low doses in patients with refractory neuroblastoma.

Methods: We performed a retrospective chart review of 10 patients with neuroblastoma treated with ^{131}I-MIBG at Rambam Health Care Campus from 1994 to 2012. Clinical data, number of ^{131}I-MIBG courses delivered, toxicities, and clinical responses were reviewed. MIBG scan was performed after each course.

Results: Twenty-one courses of ^{131}I-MIBG were delivered to 10 patients (3 girls, 7 boys). Their mean age was 3.8 years (range 1.5–6 years). All patients received several protocols of chemotherapy including the high dose form. Three patients received three courses of ^{131}I-MIBG with a minimum of 6 weeks between each course, five patients received two courses, and two patients received only one course. An objective response to the first course was obtained in nine patients and to the second course in six of eight, and in three children who underwent the third course the pain decreased. One patient has no evidence of disease, four are alive with disease, and five died of the disease. No unanticipated toxicities were observed.

Conclusions: Low dose ^{131}I-MIBG is an effective and relatively non-toxic treatment in neuroblastoma disease palliation. Rapid and reproducible pain relief with ^{131}I-MIBG was obtained in most of the children. Treatment with systemic radiotherapy in the form of low dose ^{131}I-MIBG was easy to perform and effective in cases of disseminated neuroblastoma, demonstrating that this primary therapy can be used for palliative purposes.

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KEY WORDS: neuroblastoma, palliation, ^{131}I-MIBG, radiotherapy

The choice of treatment for neuroblastoma depends on the stage of the disease, the age of the child, and biological molecular prognostic factors [1]. Despite a high initial response rate to the combination of chemotherapy, surgery, radiotherapy and immunotherapy, a large number of patients will have a recurrence of their disease before or after completion of therapy [2]. Interacting with the characteristic features of neuroblastoma, specific targeting of radiopharmaceuticals may be achieved via the metabolic route (MIBG), via receptor binding (peptides), or via the immunological route (antibodies) [1]. The active uptake mechanism in the cell membrane and neurosecretory storage granules in the cytoplasm of neuroblastoma are responsible for the uptake and retention of ^{131}I-MIBG, respectively. Although the radiopharmaceutical may be released from the granules, reuptake through this specific mechanism maintains prolonged intracellular concentration [3]. Cumulative results of ^{131}I-MIBG scintigraphy reported in the literature indicate that more than 90% of neuroblastomas concentrate ^{131}I-MIBG [4]; the uptake of ^{131}I-MIBG is tissue specific. This enables the detection of metastases regardless of their localization. Moreover, the prolonged intracellular concentration of ^{131}I-MIBG at tumor sites, in contrast to normal tissue, has led to the use of this radiopharmaceutical for therapy [5]. ^{131}I-MIBG has been used with success for radionuclide therapy of neuroblastoma since 1984 [6]. A few authors have published their experiences with higher dose ^{131}I-MIBG and demonstrated its value as a palliative agent in advanced refractory neuroblastoma [6-8]; however, thrombocytopenia limits repeated use.

Acceptable pain control can be achieved with analgesics in most children, but some patients with skeletal involvement required an alternative method of pain control during the terminal phase of their disease. We report here our experience with low dose ^{131}I-MIBG for disease palliation in refractory neuroblastoma.

PATIENTS AND METHODS [Table 1]

Patients were eligible to receive more than one course of ^{131}I-MIBG if they showed objective clinical improvement and generally decreased pain. Information collected for the study included gender, age at diagnosis, stage of disease, response