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Activity of novel lactone ketolide nafithromycin against multicentric invasive and non-invasive pneumococcal isolates collected in India

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Background: India is among the nations reporting substantial healthcare burden linked to pneumococcal infections. Nafithromycin is a novel lactone ketolide antibiotic, which recently entered Phase 3 development in India for the indication of community-acquired bacterial pneumonia (CABP).

Objectives: To assess the *in vitro* activity of nafithromycin against serotyped invasive and non-invasive *Streptococcus pneumoniae* isolates, collected from nine medical centres across India.

Methods: A total of 534 isolates of *S. pneumoniae* were collected during 2015–20 and serotyped as per CDC protocol. A subset of erythromycin-non-susceptible *S. pneumoniae* ($n = 200$) was screened for the presence of *erm*(B) and *mef*(A/E) genes. A subset of MDR isolates ($n = 54$) were also subjected to MLST. The MICs of antibiotics were determined by the reference agar-dilution method (CLSI). Susceptibilities of the comparators were interpreted as per CLSI criteria.

Results: Fifty-nine distinct serotypes were identified among the 534 isolates. Among erythromycin-non-susceptible isolates, *erm*(B) and *mef*(A/E) genes were found in 49% and 59% strains respectively, while MLST showed clonal diversity. Azithromycin (67.6% non-susceptible) and clindamycin (31.8% non-susceptible) showed limited activity. Penicillin (for non-meningitis) or quinolone non-susceptibility was low (<11% and <6%, respectively). Nafithromycin showed potent activity with MIC_{50} and MIC_{90} of 0.015–0.03 and 0.06 mg/L, respectively, regardless of the macrolide resistance mechanisms.

Conclusions: Indian pneumococcal isolates show poor susceptibilities to macrolides, in concordance with the global trend. Nafithromycin overcomes *erm* as well as *mef*-mediated macrolide resistance mechanisms expressed individually or concurrently in *S. pneumoniae*. This study supports continued clinical development of nafithromycin for pneumococcal infections including CABP.

Introduction

In India (and globally), invasive and non-invasive pneumococcal infections are accountable for a substantial healthcare burden.[1] Macrolides are 'tailor-made' antibiotics for the management of pneumococcal infections in outpatient and hospital settings for the following reasons: (i) oral bioavailability; (ii) exponential lung (site-of-infection) penetration; (iii) pharmacokinetics/pharmacodynamics (PK/PD) features permitting less frequent dosing; (iv) paediatric-use safety; (iv) favourable immune-modulating activity; and, most importantly, (v) an activity spectrum encompassing pneumonia-causing atypical bacteria.[2] However, good things don't last forever; emergence and spread of macrolide resistance mechanisms in *Streptococcus pneumoniae* has challenged the empirical utility of macrolides.[3] β-Lactams (for

non-meningococcal isolates) and quinolones still experience high susceptibility, however, the former lack coverage of atypical bacteria and their lung penetration is modest at best, while the latter, though they possess an activity spectrum suitable to monotherapy, are not considered safe in older adults and children, the most vulnerable group for pneumococcal infections. The limitations of current therapies could also be judged from the higher number of hospitalizations requiring IV therapy even among less severe pneumococcal infections.[4] Thus, an unmet need persists for a novel monotherapy that possesses potent activity against β-lactam/macrolide-resistant *S. pneumoniae*, commensurate safety profile for the target patient pool and a compliance-friendly dosing regimen (e.g. once daily, oral). Moreover, in the last 25 years, no new oral MDR pneumococci-active antibiotic has been introduced in India, despite a substantial proportion of contemporary patients presenting themselves with therapeutically challenging comorbidities and involvement of resistant pathogens.

Nafithromycin is a novel lactone ketolide (advanced generation macrolide), presently under clinical development for community-acquired bacterial pneumonia (CABP). It overcomes all three of the macrolide resistance mechanisms—*Erm*, efflux and ribosomal protein mutations—in *S. pneumoniae*.[5] In a global surveillance study, nafithromycin showed potent activity against respiratory pathogens including macrolide-resistant *S. pneumoniae*.[5] Moreover, it retains consistent activity against β-lactam and quinolone-resistant *S. pneumoniae* and covers atypical respiratory pathogens. The Phase 1 pulmonary pharmacokinetic study revealed high and sustained lung epithelial lining fluid (ELF) exposures of nafithromycin (69× higher than the plasma unbound exposures). Moreover, compared with plasma unbound exposures, concentrations of nafithromycin in alveolar macrophages are significantly higher (2635×), which is expected to deliver high drug concentrations at the site of infection and also facilitate intracellular killing of the pathogen.[6] The higher pulmonary exposures facilitate attainment of PK/PD targets demonstrated through Monte-Carlo simulation and ensures efficacy of 3 day, once daily (OD) dosing regimen of nafithromycin for CABP. A global Phase 2 study, comparing a 3 day OD regimen of nafithromycin with a 7 day OD regimen of moxifloxacin, has established safety and efficacy of nafithromycin (ClinicalTrials.gov identifier NCT02903836). A Phase 3, randomized, double-blinded, non-inferiority study has been initiated to assess the safety and efficacy of 3 day OD treatment of nafithromycin in Indian adult CABP patients. Ahead of this study, we investigated the nafithromycin's *in vitro* activity against contemporary invasive and non-invasive *S. pneumoniae* isolates collected from major Indian cities.

Materials and methods

Bacterial strains

A total of 534 non-duplicate (one isolate per patient) *S. pneumoniae*, isolated from sterile sites—blood ($n = 352$), cerebrospinal fluid ($n = 30$), pleural fluid ($n = 52$)—as well as from sputum ($n = 100$) were included. These isolates were collected during 2015–20, from Christian Medical College, Vellore [WHO regional reference laboratory for *S. pneumoniae* in South East Asia ($n = 316$)], from New Delhi [Chacha Nehru Bal Chikitsalaya ($n = 106$)], Chennai [Kanchi Kamakoti Children's Trust Hospital ($n = 59$) and Institute of Child Health ($n = 10$)], Mumbai [Joshi's Lab ($n = 11$) and Lokmanya Tilak Municipal General Hospital and Medical College ($n = 5$)], Hyderabad [Global Hospitals ($n = 15$)], Bengaluru [Institute of Child Health $n = 7$)] and Jodhpur [All India Institute of Medical Sciences ($n = 5$)], all located in India. Isolates were identified by a protocol described by CDC, USA.[7] Further, isolates were serotyped by CDC recommended Quellung reaction using type-specific pneumococcal antisera obtained from Statens Serum Institut and also by a customized sequential multiplex PCR as previously described.[8]

Identification of genes conferring macrolide resistance

A randomly selected subset of erythromycin-non-susceptible *S. pneumoniae* ($n = 200$) were screened for the presence of *erm*(B) and *mef*(A/E) by PCR method.[9]

Sequence typing

A randomly selected 54 MDR [non-susceptible to erythromycin and penicillin G (meningitis criteria)] invasive *S. pneumoniae* were subjected to MLST.[10]

MIC determination

The MICs were determined by reference agar dilution as recommended by CLSI (M07, 2019). Nafithromycin was synthesized at Wockhardt Research Centre (97.3% purity). Other antibiotics were from commercial sources. Quality control strains *S. pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 25923 were included in each run. The susceptibilities were interpreted against CLSI criteria (M100, 2020).

Results

Serotypes

Fifty-nine distinct serotypes were found in the 534 isolates; dominants (≥40 isolates) were 19 F ($n = 65$), 14 ($n = 59$) and 9 V ($n = 47$). In invasive isolates, serotype coverage of PCV13 and PPSV23 vaccines were 36% and 41% respectively, which improved to 54% and 64%, for non-invasive isolates.

Identification of genes conferring macrolide resistance

Among the genetically characterized 200 erythromycin-non-susceptible isolates, *erm*(B) and *mef*(A/E) were found in 49% and 59% of isolates respectively; 10% of isolates harboured both *erm*(B) and *mef*(A/E).

Sequence types

The MLST revealed the existence of 34 diverse STs and 16 clonal complexes (CCs) in the 54 invasive MDR isolates analysed. The most prevalent CCs were CC320 ($n = 12$), CC63 ($n = 8$) and CC156 ($n = 6$).

MICs

Table 1 shows the nafithromycin MIC distribution for all *S. pneumoniae* and antibiotic-resistant subsets. Nafithromycin showed a potent activity, with MICs being in a narrow range (MIC_{50} and MIC_{90} of ≤0.03 mg/L and ≤0.06 mg/L) regardless of resistance subsets. Table 2 provides the comparative activity of nafithromycin with other anti-pneumococcal antibiotics. The tested population showed low susceptibilities to macrolides: 26.6% for erythromycin and 32.4% for azithromycin, while clindamycin susceptibility was at 68.2%. A high susceptibility (>89%) was observed for penicillin G, amoxicillin/clavulanate, cefotaxime (non-meningitis) and

Table 1. Nafithromycin MIC distribution for *S. pneumoniae* isolates

	Number of isolates inhibited at each concentration (% isolates cumulatively inhibited)							MIC (mg/L)	
	≤0.007	0.015	0.03	0.06	0.12	0.25	0.5	50%	90%
All ($n = 534$)	89 (16.7)	173 (49.1)	176 (82)	77 (96.4)	16 (99.4)	2 (99.8)	1 (100)	0.03	0.06
ERY-S ($n = 142$)	52 (37)	47 (69.7)	36 (95)	7 (100)				0.015	0.03
ERY-R ($n = 355$)	23 (6.5)	116 (39.2)	129 (75.5)	68 (94.6)	16 (99.2)	2 (99.7)	1 (100)	0.03	0.06
CLI-R ($n = 163$)	10 (6.1)	49 (36.2)	59 (72.4)	32 (92)	11 (98.8)	1 (99.4)	1 (100)	0.03	0.06
erm(B)[a] ($n = 98$)	12 (12.2)	36 (49)	36 (85.7)	13 (99)	1 (100)			0.03	0.06
mef(A/E)[b] ($n = 118$)	5 (4.2)	46 (43.2)	50 (85.6)	15 (98.3)	2 (100)			0.03	0.06
LVX-R ($n = 28$)	5 (17.9)	10 (53.6)	8 (82.1)	5 (100)				0.015	0.06

ERY-R, erythromycin resistant (MICs ≥1 mg/L); CLI-R, clindamycin resistant (MICs ≥1 mg/L); LVX-R, levofloxacin resistant (MICs ≥4 mg/L).
[a]A total of 98 out of 200 randomly characterized erythromycin-non-susceptible isolates carried *erm*(B).
[b]A total of 118 out of 200 randomly characterized erythromycin-non-susceptible isolates carried *mef*(A/E).

Table 2. Activity of nafithromycin and other comparator antibiotics against *S. pneumoniae* isolates

	MIC (mg/L)			
Antibiotic	range	MIC_{50}	MIC_{90}	Percentage susceptible[a]
Nafithromycin	≤0.007 to 0.5	0.03	0.06	NA
Erythromycin	≤0.06 to >32	2	>32	26.6
Azithromycin	≤0.03 to >32	2	>32	32.4
Clindamycin	≤0.06 to >32	≤0.06	>32	68.2
Levofloxacin	0.03 to >8	1	2	94.8
Moxifloxacin	≤0.015 to 8	0.12	0.25	94.6
Penicillin G	≤0.03 to 8	0.5	4	30.1[b], 89.1[c]
Cefotaxime	≤0.015 to 8	0.25	1	73.8[b], 93.6[c]
Amoxicillin/clavulanic acid	≤0.015/0.007 to >8/4	0.25/0.125	2/1	91.6[c]

NA, not applicable.
[a]CLSI interpretive criteria.
[b]Meningitis criteria.
[c]Non-meningitis criteria.

quinolones. Penicillin G and cefotaxime susceptibilities based on meningitis criteria were 30.1% and 73.8%, respectively.

Discussion

For a long time, macrolides have rightfully been a preferred treatment for CABP and other pneumococcal infections due to (i) convenience in dosing and (ii) a broad-spectrum pathogen coverage assuring the success of monotherapy. However, rising macrolide resistance has significantly compromised their monotherapy potential, compelling clinicians to combine macrolides with β-lactams, as a compromised treatment strategy.[11] Such combinations pose their own challenges in terms of patient compliance, safety and the risk of selection and proliferation of clones resistant to both macrolides and β-lactams. In the USA and Europe, clones of macrolide-resistant *S. pneumoniae* co-harbouring penicillin resistance are emerging, thus rendering the two mainstay therapies unfeasible.[12] Lack of reliable therapeutic options in the community leads to an increase in avoidable hospitalization, thus escalating the treatment cost, exerting pressure on already scarce hospital resources and exposing the patients to healthcare-associated infections.[13]

Emerging therapeutic profile of nafithromycin indicates that upon successful development it has a potential to offer a viable monotherapy with a compliance-friendly, 3 day regimen. The present study was undertaken to assess the current level of susceptibility to nafithromycin ahead of its Phase 3 study and establish baseline susceptibility rates in *S. pneumoniae* to help monitor any future shifts.

The current panel of multicentre Indian pneumococcal isolates showed high rates of macrolide non-susceptibility (>60%), which is in line with a previous report.[14] The higher rate of susceptibility to clindamycin as compared with macrolides suggests a significant prevalence of efflux as a macrolide-impacting resistance mechanism.[15] Among the 200 genetically screened isolates, both *erm* and *mef*(A/E), were almost equally prevalent. In contrast, Erm and Mef are the dominant resistance mechanisms in China and the USA, respectively.[16,17] Quinolones were active with >94% susceptibility

and penicillin G susceptibilities were contingent on the breakpoints used, with low susceptibility for meningitis and higher susceptibility for pneumonia. The analysis revealed existence of non-vaccine serotypes in significant proportions, indicating the collateral effect of vaccination-triggered serovar replacements.

The MIC profile of nafithromycin obtained in this study (MIC_{90} of 0.06 mg/L) is in concordance with the previous global surveillance study and demonstrates its potent activity against macrolide, penicillin and quinolone-non-susceptible pneumococci.[5] Moreover, this MIC_{90} is well within the coverage profile of nafithromycin as per the Monte-Carlo simulation and probability of target attainment analyses.[18] Having demonstrated consistent activity against Indian isolates harbouring globally prevalent *erm* and *mef*(A/E), nafithromycin is expected to show a comparable activity against pneumococci from lower-middle-income countries.

Tightly clustered MICs of nafithromycin against *erm*(B)-harbouring *S. pneumoniae* possibly indicate nafithromycin's high affinity for the methylated ribosomal targets. This observation is supported by a previous report describing its superior inhibitory activity against *S. pneumoniae* subjected to a higher level of *erm*(B) induction through exposure to subinhibitory erythromycin, which is ascribed to its favourable interaction with domain II in addition to the conventional macrolide target of domain V.[19] Under similar conditions, cethromycin, telithromycin and solithromycin showed significant upward shift in MICs due to *erm*(B) hyper-induction.[20]

There are a few limitations in this study: among 392 erythromycin-non-susceptible isolates, only 200 were analysed for the presence of *erm*(B) and *mef*(A/E) and MLST was not performed for non-invasive isolates.

In summary, the nafithromycin's features such as potent activity against *S. pneumoniae* and other respiratory pathogens, high and sustained lung concentrations and reported anti-inflammatory/immunomodulatory effects[21] point towards its promising therapeutic potential in the management of CABP infections.

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Transparency declarations

None to declare.

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