FLUOROQUINOLONE, MACROLIDE AND KETOLIDE RESISTANCE IN HAEMOPHILUS PARAINFLUENZAE FROM THE PRIVATE HEALTH SECTOR IN DURBAN, SOUTH AFRICA

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INTRODUCTION:

H. parainfluenzae (HP) is a Gram-negative bacteria implicated in the etiology of pneumonia and infective endocarditis [1]. Fluoroquinolones, macrolides and ketolides are antibiotics of choice in resolving infections caused by beta-lactam resistant strains of HP. However, resistance to these antibiotics is increasingly being reported in this species [2,3] and not in *H. influenzae* in the private sector of KwaZulu-Natal (KZN) and Gauteng Provinces of South Africa [4].

PURPOSE:

This study described resistance trends to selected antibiotics from 2012 to 2015 in HP and further genotypically characterized fluoroquinolone, macrolide and ketolide resistance in a selected sub-sample, delineating mutations (if evident) in common resistance genes.

METHODS:

Antibiotic susceptibility data from January 2012 to June 2015 for *H. parainfluenzae* (n = 4803) were retrieved from a private laboratory database and analyzed. Ten HP isolates resistant to one or more of the fluoroquinolones, macrolides and ketolides, prospectively collected from April to June 2015 were subjected to sensitivity testing using the CLSI broth micro-dilution method, PCR and DNA sequencing of selected resistance genes and repetitive extragenic palindromic PCR to ascertain clonality.

RESULTS:

- *H. parainfluenzae* resistance to fluoroquinolones increased from 4.1% (n=1048) in 2012 to 21.4% (n=816) in 2015 [p<0.0002] and from 8.4% to 15.9% [p<0.0001] for telithromycin but decreased from 14% to 9.3% [p<0.0002] for ampicillin.
- Fluoroquinolone resistance was attributed to the amino acid substitutions S84F and D88Y in *gyrA*, S84Y, S138T, M198L and the novel S84L change in *parC* gene. No mutations were detected in the *gyrB* gene.
- The plasmid-mediated-quinolone-resistance gene *aac- (6')-Ib-cr* was detected for the first time in 4 isolates of *H. parainfluenzae* globally and D420N change was observed in *par E* in one isolate.
- Macrolide and ketolide resistance was ascribed to the resistance genes, *mefA* and the novel *msrD* and *ermB* genes as well as A69S substitution in L4 ribosomal protein detected.
- REP-PCR analysis showed that the isolates were unrelated.
- All the observed resistance mechanisms are first reports in Africa.

CONCLUSION:

There is an emerging fluoroquinolone and macrolide/ketolide resistance in *H. parainfluenzae* isolates from the private sector patients in South Africa attributable to known/novel resistance mechanisms necessitating the monitoring of this pathogen as a potential opportunistic pathogen in a country with a high HIV and AIDS prevalence.

Table 2. MIC and Mechanisms of Resistance to Fluoroquinolones, Macrolides, and a Ketolide in *H. parainfluenzae* Isolates

	MIC (mg/L)						Resistance Mechanisms					
							Macrolides		Fluoroquinolones			
Isolate	**[1]NAL**	**[2]CIP**	**[3]GAT**	**[4]ERY**	**[5]AZM**	**[6]TEL**	**L4**	**Acquired**	***Gyr A***	***Par C***	***Par E***	**[8]PMQR**
RK 21	256	8	8	128	64	32	A69S*		S84F,D88Y	S84Y, S138T, M198L	[9]NC	
RK 24	128	16	16	512	>256	128	A69S*	*Mef* (A), *Msr* (D)	S84F, D88Y	S84Y, S138T, M198L	NC	
RK 25	128	16	16	256	128	32	A69S*		S84F, D88Y	S84Y, S138T,M198L	NC	
RK 26	256	8	16	16	4	2	A69S*		S84F, D88Y	S84Y, S138T, M198L	NC	
RK 29	128	8	16	256	32	32	A69S*		S84F, D88Y	S84Y, S138T, M198L	NC	*[10]Aac- (6)*
RK 33	>512	4	4	512	>256	>512	A69S*	*Erm* (B)	S84F, D88Y	S84L, M198L	NC	
RK 34	512	32	32	2	0.5	0.25	A69S*		S84F, D88Y	S84Y,S138T,M198L	NC	
RK 39	128	8	16	256	128	16	A69S*		S84F, D88Y	S84Y, S138T, M198L	D420N	*[10]Aac-(6')*
RK 40	256	32	32	512	>256	128	A69S*	*Mef* (A), *msr* (D)	S84F, D88Y	S84Y, S138T, M198L	NC	*[10]Aac-(6')*
RK 41	128	8	16	256	256	32	A69S*		S84F, D88Y	S84Y, S138T, M198L	NC	*[10]Aac-(6')*
[7]CTRL	1	0.008	0.0156	1	4	4						

* The Ala69Ser substitution was observed after comparison with *H. influenzae* Rd but not with *H. parainfluenzae* T3T1. [1]Nalidixic acid, [2]Ciprofloxacin, [3]Gatifloxacin,[4]Erythromycin, [5]Azithromycin. [6]Telithromycin, [7]ATCC 49247, [8]Plasmid-Mediated Quinolone Resistance gene, [9]No change, [10]Aac-(6')-Ib-cr. All isolates were recovered from the sputum of patients with *H. parainfluenzae* pneumonia.

Table 1. Percentage resistance of *H. parainfluenzae* isolates collected over 42-month period in the private sector of KwaZulu-Natal and Gauteng provinces.

Year	2012	2013	2014	2015	[a]P values	Statistically significant? (alpha < 0.05)
Antibiotic	Percentage Resistance					
Ampicillin	14	12.6	10.9	9.3	<0.0002	Yes
Ciprofloxacin	4.1	9.3	18	21.4	<0.0002	Yes
Telithromycin	8.4	7.3	9.1	15.4	<0.0001	Yes
Total No. Isolates	1048	1275	1664	816		

[a]P value based on Chi-square test for trend determined using Graphpad prism 5 version 5.01 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com

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