

Blackman Northwood J¹, C. Couturier², S. Brown³, D. Knight¹, I. Morrissey^{1*}

¹Quotient Bioresearch Ltd, Microbiology, Cambridgeshire, UK. ²Sanofi aventis, Paris, France. ³Clinical Microbiology Institute, Portland, USA..

Abstract

Objective: The genetic & geographical relationship between quinolone-resistant (QR) *S. pneumoniae* (SP) collected as part of PROTEKT Global & PROTEKT US was determined.

Methods: SP were collected from PROTEKT Global (47 countries worldwide; N=41,693) & PROTEKT US (49 states; N=55,729). MICs were determined using CLSI guidelines. Quinolone resistance determining regions (QRDR) within *gyrA*, *gyrB*, *parC* & *parE* were sequenced & multi locus sequence typing determined for QRSP by previously published methods.

Results: QR rates for both studies remained stable ~1% each year (Table 1). However, Italy had a steadily increasing rate of QR. Hong Kong had a very high QR rate while Massachusetts (MA), Colorado (CO), New Jersey (NJ) & New York (NY) had higher than the US average (0.9% [SD0.6]) QR rates. Globally, QRSP were from a wide range of sequence types (ST) & clonal complexes (CC). QRSP from Hong Kong were mainly ST81 (58/71), which was found in numerous locations worldwide. ST66 was the dominant clone in Italy (58/81), most collected from a single centre. There were 3 dominant CC in the US: CC81 (67/527), CC271 (37/527) & CC439 (34/527). QRSP were represented by >23 CC & were evenly distributed across the US except for CC81 which peaked in NY & CC439 which peaked in MA. QR was due to typical QRDR mutations as previously reported.

Conclusion: QR in Hong Kong was due to the dominance of CC81, as previously reported. QR in Italy was due to the emerging resistant clone ST66, although this could be a localized outbreak as most came from 1 centre in Catania over a short period of time. QRSP in the US was due to the presence of numerous clones including CC81 & CC439. The presence of CC271 in QRSP is also important as this clone is often associated with resistance to macrolides, penicillin, tetracycline & cotrimoxazole. This study shows QR remained very low during both studies & was caused by multiple CC in most countries.

Introduction

- *S. pneumoniae* isolates have shown increased resistance to numerous classes of antimicrobials [1].
- Quinolone resistance is uncommon in SP[2].
- Quinolones inhibit bacterial DNA synthesis by targeting the DNA gyrase and topoisomerase IV proteins, essential for DNA replication.
- Quinolone resistance in *S. pneumoniae* is mediated by stepwise mutations in QRDRs of the respective genes and occurs more commonly in *parC* and *gyrA* [3].
- The aim of this study was to characterise the genetic and geographical relationship between quinolone resistant *S. pneumoniae*.

Methods

- Isolates were collected from 47 countries worldwide (N=41,693) as part of PROTEKT Global between 2000 and 2007.
- Additionally, 55,729 isolates were collected from 49 states as part of PROTEKT US between 2000 and 2006.
- Minimum Inhibitory concentrations (MIC) were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [4].

Methods (contd)

- Sequencing analysis of the QRDR within *gyrA*, *gyrB*, *ParC* and *ParE* genes and multi locus sequence typing of *S. pneumoniae* was performed according to previously published methods [5, 6].

Results

- Quinolone resistance rates for *S. pneumoniae* remained at approximately 1% each year for both projects.
- Italy had a steadily increasing rate of quinolone resistance.
- Hong Kong had a very high rate of quinolone resistance.
- MA, CO, NJ and NY had quinolone resistance rates above the US average of 0.9% [SD0.6].
- Globally, quinolone resistant *S. pneumoniae* were from a wide range of sequence types and clonal complexes.
- ST81 was the predominant quinolone resistant *S. pneumoniae* clone in Hong Kong (58/71) and was also found in numerous other locations worldwide.
- Quinolone resistant *S. pneumoniae* from Italy were mainly ST66 (58/81).
- CC81 (67/527), CC271 (37/527) & CC439

Results (contd)

- (34/527) were the dominant clones amongst greater than 23 clonal complexes identified in the US.
- Clonal complexes were evenly distributed across the states except for CC81 and CC439 which had peaks in frequency in NY and MA, respectively.
- Quinolone resistance was due to typical QRDR mutations as previously reported.

Conclusions

- Dominance of CC81 in Hong Kong was the cause of the high quinolone resistance, as previously reported [2].
- Quinolone resistance in Italy was due to the emerging resistant clone ST66, although most of these isolates were collected from a single centre within a short period of time and therefore may be a localised outbreak.
- Quinolone resistant *S. pneumoniae* in the US was due to the presence of numerous clones including CC81, CC271 & CC439.
- The presence of CC271 in quinolone resistant *S. pneumoniae* is also important as this clone is often associated with resistance to macrolides, penicillin, tetracycline & cotrimoxazole.
- This study shows quinolone resistance remained very low during both studies & was caused by multiple clonal complex in most countries.

References

1. Felmingham, D., and J. Washington. 1999. J Chemother 11 Suppl 1:5-21.
2. Canton, R., M. Morosini, M. C. Enright, and I. Morrissey. 2003. J Antimicrob Chemother 52:944-52.
3. Gootz, T. D., R. Zaniewski, S. Haskell, B. Schmieder, J. Tankovic, D. Girard, P. Courvalin, and R. J. Polzer. 1996. Antimicrob Agents Chemother 40:2691-7.
4. CLSI. (Current in year of testing). Clinical and Laboratory Standards Institute document M7. Clinical and Laboratory Standards Institute, Wayne, PA.
5. Morrissey, I., A. Colclough, and J. Northwood. 2007. Int J Antimicrob Agents 30:345-51.
6. Enright, M. and Spratt, B.G.(1998) Microbiology 144, 3049-3060.

Table 1. Total number of isolates and percentage resistant by region

		Total number of isolates collected (N) & % QR by Year [NT; not tested]							
Region		2000-01	01-02	02-03	03-04	04-05	05-06	06-07	Average
Hong Kong	N	70	58	74	70	62	73	93	71
	%QR	14.3%	15.5%	10.8%	11.4%	17.7%	17.9%	12.9%	14.2%
Italy	N	119	284	267	285	228	192	590	281
	%QR	0.0%	0.7%	1.5%	3.9%	4.4%	5.2%	7.5%	4.1%
MA	N	353	268	246	205	137	106	NT	219
	%QR	4.8%	1.9%	1.2%	2.4%	2.2%	0.0%	NT	2.5%
CO	N	65	239	265	108	226	167	NT	178
	%QR	4.6%	4.2%	2.6%	0.9%	0.9%	0.6%	NT	2.2
NJ	N	191	193	160	35	81	NT	NT	132
	%QR	1.0%	3.1%	1.9%	2.9%	0.0%	NT	NT	1.8
NY	N	542	529	597	493	626	548	NT	556
	%QR	1.7%	1.1%	3.0%	1.6%	1.4%	1.3%	NT	1.7%
Global Average	N	3435	4256	6320	6739	7083	6395	7465	-
	%QR	1.4%	1.1%	0.9%	1.0%	1.0%	1.4%	1.4%	1.1%
US	N	10,103	10,012	10,886	8494	9487	6747	NT	-
	%QR	0.9%	1.2%	0.8%	1.1%	0.9%	0.8%	NT	0.9%

*Presenting author: ian.morrissey@quotientbioresearch.com. Quotient Bioresearch Ltd. Newmarket Road, Fordham, Cambridgeshire CB7 5WW