Infection, Genetics and Evolution 31 (2015) 68–72

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Clonal distribution of pneumococcal serotype 19F isolates from Ghana

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ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 16 January 2015
Accepted 20 January 2015
Available online 29 January 2015

Keywords:
Streptococcus pneumoniae
Serotype
19F
Clone
Ghana
Carriage

ABSTRACT

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide. Pneumococcal strains are classified according to their capsular polysaccharide and more than 90 different serotypes are currently known.

In this project, three distinct groups of pneumococcal carriage isolates from Ghana were investigated; isolates from healthy children in Tamale and isolates from both healthy and children attending the outpatient department at a hospital in Accra. The isolates were previously identified and characterized by Gram staining, serotyping and susceptibility to penicillin. In this study, isolates of the common serotype 19F were further investigated by Multi-Locus Sequence Typing (MLST).

Overall, 14 different Sequence Types (STs) were identified by MLST, of which nine were novel based on the international MLST database. Two clones within serotype 19F seem to circulate in Ghana, a known ST (ST 4194) and a novel ST (ST 9090). ST 9090 was only found in healthy children in Accra, whereas ST 4194 was found equally in all children studied. In the MLST database, other isolates of ST 4194 were also associated with serotype 19F, and these isolates came from other West African countries. The majority of isolates were penicillin intermediate resistant.

In conclusion, two clones within serotype 19F were found to be dominating in pneumococcal carriage in Accra and Tamale in Ghana. Furthermore, it seems as though the clonal distribution of serotype 19F may be different from what is currently known in Ghana in that many new clones were identified. This supports the importance of continued monitoring of pneumococcal carriage in Ghana and elsewhere when vaccines, e.g., PCV-13, have been introduced to monitor the possible future spread of antimicrobial resistant clones.

1. Introduction

With the help of the GAVI Alliance (formerly the Global Alliance for Vaccines and Immunization) and the World Health Organization (WHO), vaccination programs have been introduced in Africa. These include pneumococcal conjugate vaccines such as the 10-valent pneumococcal *Haemophilus influenzae* conjugate vaccine (PCV-10, GlaxoSmithKline) and a 13-valent pneumococcal conjugate vaccine (PCV-13, PrevnarTM, Pfizer Vaccines) (Mulholland and Satzke, 2012; O'Brien, 2013; www.gavialliance.org). However, despite increased research interest in *Streptococcus pneumoniae* in Africa (Mulholland and Satzke, 2012; O'Brien, 2013) the epidemiology of *S. pneumoniae* carriage and the associated disease is both complex and dynamic. Many factors affect pneumococcal carriage including serotype distribution which varies with geographical location, ethnicity, immunosuppression, vaccination, antimicrobial consumption and age (Harboe et al., 2010; Holliman et al., 2007).

Ghana, a West African country, has recently introduced the PCV-13 in their childhood vaccination program (Dayie et al., 2013). Knowledge on the pneumococcal carriage and serotype distribution are important when vaccines are introduced, e.g., in Ghana (Dayie et al., 2013; Donkor et al. 2010). A recent study on the pneumococcal carriage in Ghana revealed that the most common serotypes in children were 19F, 6B, 23F, and 6A, of which serotype 19F was the predominant serotype observed both in the capital city of Accra and in Tamale, a large city in the northern part of the country (Dayie et al., 2013). Serotype 19F is a common

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http://dx.doi.org/10.1016/j.meegid.2015.01.016
1567-1348/

Table 1
MLST of 48 *Streptococcus pneumoniae* serotype 19F isolates including ST number, allele No. and MIC values to penicillin (μg/mL). Bold numbers indicate new STs and alleles. Isolates that are sensitive and intermediate resistant to penicillin are illustrated with grey and white rows, respectively.

Origin	Isolate name	ST	*aroE*	*gdh*	*gki*	*recP*	*spi*	*xpt*	*ddl*	Penicillin MIC (mg/L)
Accra	JAS7	2520	7	16	8	8	6	142	235	0.25
Accra	ACZ18	**9086**	2	5	8	10	17	1	31	0.25
Accra	HAK32	4194	2	5	9	10	17	1	31	0.25
Accra	HAK92	**9087**	1	134	101	18	36	**527**	18	0.016
Accra	AAK19	**9088**	12	19	2	6	6	22	13	0.064
Accra	AAK12	4194	2	5	9	10	17	1	31	0.25
Accra	WCD1	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCD27	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCF10	**9089**	2	25	9	10	17	1	31	0.032
Accra	WCF6	4194	2	5	9	10	17	1	31	0.25
Accra	WCD55	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCD54	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCD53	4194	2	5	9	10	17	1	31	0.25
Accra	WCD51	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCD50	**9090**	7	13	8	8	6	142	235	0.032
Accra	WCD47	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCD46	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCD45	**9090**	7	13	8	8	6	142	235	0.125
Accra	WCD41	**9090**	7	13	8	8	6	142	235	0.25
Accra	AEK19	180	7	15	2	10	6	1	22	0.125
Accra	WCF12	4194	2	5	9	10	17	1	31	0.25
Accra	WCD61	**9090**	7	13	8	8	6	142	235	0.125
Hospital	A56	4194	2	5	9	10	17	1	31	0.125
Hospital	A80	4194	2	5	9	10	17	1	31	0.125
Hospital	A138	4194	2	5	9	10	17	1	31	0.125
Hospital	A143	4194	2	5	9	10	17	1	31	0.25
Hospital	B114	**9092**	50	5	123	1	36	83	14	0.25
Hospital	B33	4194	2	5	9	10	17	1	31	0.125
Hospital	B37	**9093**	1	25	101	18	36	1	18	0.032
Hospital	B66	**9094**	60	134	367	4	42	3	6	0.047
Hospital	B97	4194	2	5	9	10	17	1	31	0.25
Hospital	B124	4194	2	5	9	10	17	1	31	0.125
Hospital	B130	4194	2	5	9	10	17	1	31	0.125
Hospital	B131	802	10	13	53	1	72	38	31	0.016
Hospital	D63	4194	2	5	9	10	17	1	31	0.125
Tamale	MK54	4194	2	5	9	10	17	1	31	0.25
Tamale	KP62	4194	2	5	9	10	17	1	31	0.125
Tamale	MK68	8152	13	9	4	18	17	7	9	0.25
Tamale	MK63	4194	2	5	9	10	17	1	31	0.125
Tamale	BB17	4194	2	5	9	10	17	1	31	1.0
Tamale	KP6	4194	2	5	9	10	17	1	31	0.125
Tamale	MK96	4194	2	5	9	10	17	1	31	0.125
Tamale	MK113	4194	2	5	9	10	17	1	31	0.25
Tamale	LM25	4194	2	5	9	10	17	1	31	0.25
Tamale	KP38	4194	2	5	9	10	17	1	31	0.064
Tamale	KP4	**9091**	2	89	9	38	6	12	18	0.25

serotype observed in many countries, and it is also included in the commercially available pneumococcal vaccines (Harboe et al., 2009; Li et al., 2013; Nathan et al., 2014).

The aim of this study was to use Multi Locus Sequence Typing (MLST) of serotype 19F pneumococcal carriage isolates collected by Dayie et al. (2013) and carriage isolates from children attending the outpatient department at the Princess Marie Louise Children's Hospital in Accra to identify predominant clonal complexes, association between certain MLST types and the level of penicillin resistance and to determine differences in isolates from Accra and Tamale.

2. Materials and methods

2.1. S. pneumoniae isolates

A total of 48 carriage *S. pneumoniae* serotype 19F isolates were selected including 22 isolates from Accra and 13 isolates from Tamale (Dayie et al., 2013). The isolates (35) originated from the nasopharynges of healthy children less than six years of age attending random selected kindergartens and nursery schools in Accra (11 sites) and Tamale (7 sites) (Dayie et al., 2013). Furthermore, 13 *S. pneumoniae* serotype 19F isolates were selected among a collection of 207 carriage *S. pneumoniae* isolates collected from children attending the outpatient department at the Princess Marie Louise Children's Hospital in Accra (kindly provided by Richael Mills). All isolates studied were collected in 2011 before the introduction of PCV-13 vaccine in the spring of 2012 (www.gavialliance.org).

Characteristics of the 35 *S. pneumoniae* serotype 19F isolates from Accra and Tamale including serotype and penicillin susceptibility have previously been determined by Dayie et al. (2013) and confirmed in this study as shown below. The 13 *S. pneumoniae* serotype 19F isolates from children seen at the Princess Marie Louise Children's Hospital were only serotyped previously (Richael Mills, personal communication).

In the current study, the identification of all 48 isolates were reconfirmed by Gram staining, optochin susceptibility, bile solubility and their serotype 19F status. Penicillin resistance was recon-