NOVEL SEROGROUP 24 ISOLATES FROM INVASIVE PNEUMOCOCCAL DISEASE IN GERMANY

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BACKGROUND AND AIMS

The German National Reference Center for Streptococci has been analyzing invasive pneumococcal isolates since 1992. Six isolates in our collection were not serologically consistent with the long-recognized serotypes 24F, 24A, and 24B. Here we describe the genetic and serological characteristics of these isolates.

METHODS

771 serogroup 24 isolates were identified by Neufeld-Quellung reaction at the German National Reference Center for Streptococci from January 1, 1992 to June 30, 2017. Six of these isolates could only be identified as Serogroup 24. Pulsed Field Gel Electrophoresis (PFGE), Comparative Genomic Hybridization Microarray, Multilocus Sequence Typing (MLST), and Whole Genome Sequencing (WGS) were performed on these isolates..

RESULTS

Serogroup 24 consists of the serotypes 24F, 24A and 24B. The genetic organization of serotype 24A differs from 24F and 24B in that the *wzy* and *rbsF* genes are misssing. Differences between 24F and 24B are more subtle (**Fig. 1**).

All of the isolates were identified as 24F by microarray, but this was not consistent with serological results. Serological results showed that both factorserum 24d and factorserum 24e were positive, whereas 24d is specific for serotype 24F and 24e is specific for 24B. This indicates that the six isolates have immunological characteristics of both serotypes 24F and 24B (**Table 1**).

PFGE results indicate that each isolate has an individual banding pattern which are all unlike the control strains for 24A, 24B and 24F (**Fig. 2**).

MLST results showed isolates from ST72 (often found in serotype 24F) and ST 162 (found in serotype 24F as well as several other serotypes; **Table 2**). PFGE patterns are more similar to each other within one sequence type, than to the other sequence type.

We have obtained whole genome sequences of all 6 isolates and have compared the capsular cassettes to those of 24F, 24A and 24B.

The whole genome sequences of the isolates grouped in two groups: isolates 2,3,5 and isolates 6,7,8. This was in correlation with the MLST results. Capsular cassettes were identical within each group (and also between both groups) and one representative of each ST was further analyzed (Isolates 2 and 7).

The gene organization within the capsular cassettes showed the presence of the *wzy* and *rbsF* genes, as in seroytpes 24F and 24B. This correlates with the serological data.

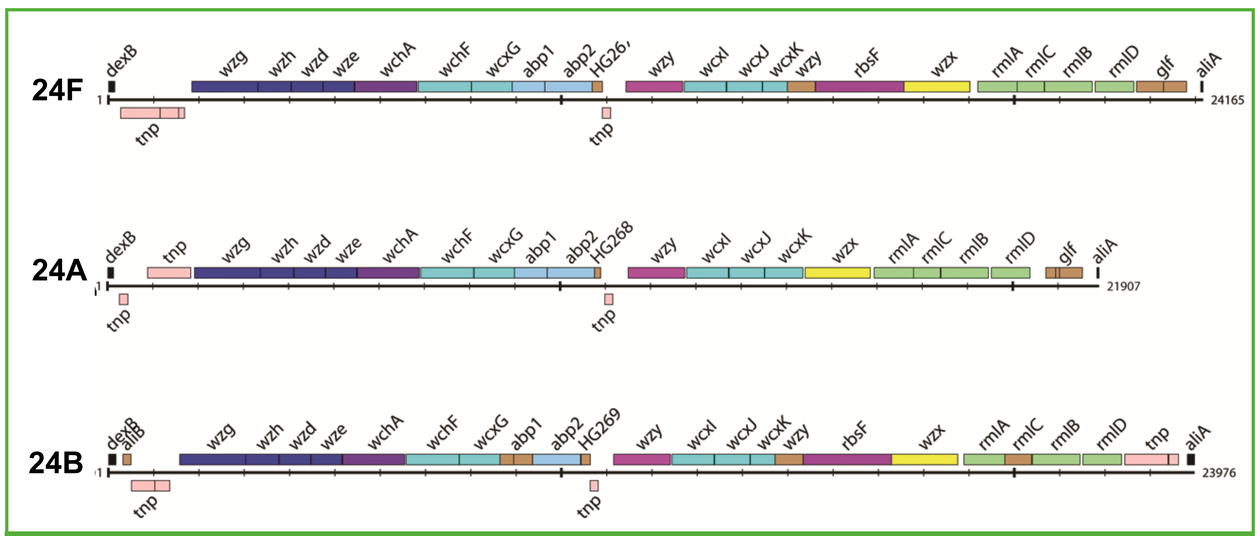

Fig 1: Genetic organization of the three serotypes in serogroup 24 (Mavroidi et al., J Bacteriol. 2007 Nov;189(21):7841-55).

Table 1: Serotyping results using the 'Neufeld'sche Quellung Methode' of Serogroup 24 isolates with unusual factorserum reactions.

Isolates	Poolserum C	Groupserum 24	Factorserum 24c	Factorserum 24d	Factorserum 24e	Serotype
Reference 24F		+	-	+	-	24F
Reference 24A		+	+	-	-	24A
Reference 24B		+	-	-	+	24B
Isolate 2	+	+	-	+	+	24?
Isolate 3	+	+	-	+	+	24?
Isolate 5	+	+	-	+	+	24?
Isolate 6	+	+	-	+	+	24?
Isolate 7	+	+	-	+	+	24?
Isolate 8	+	+	-	+	+	24?

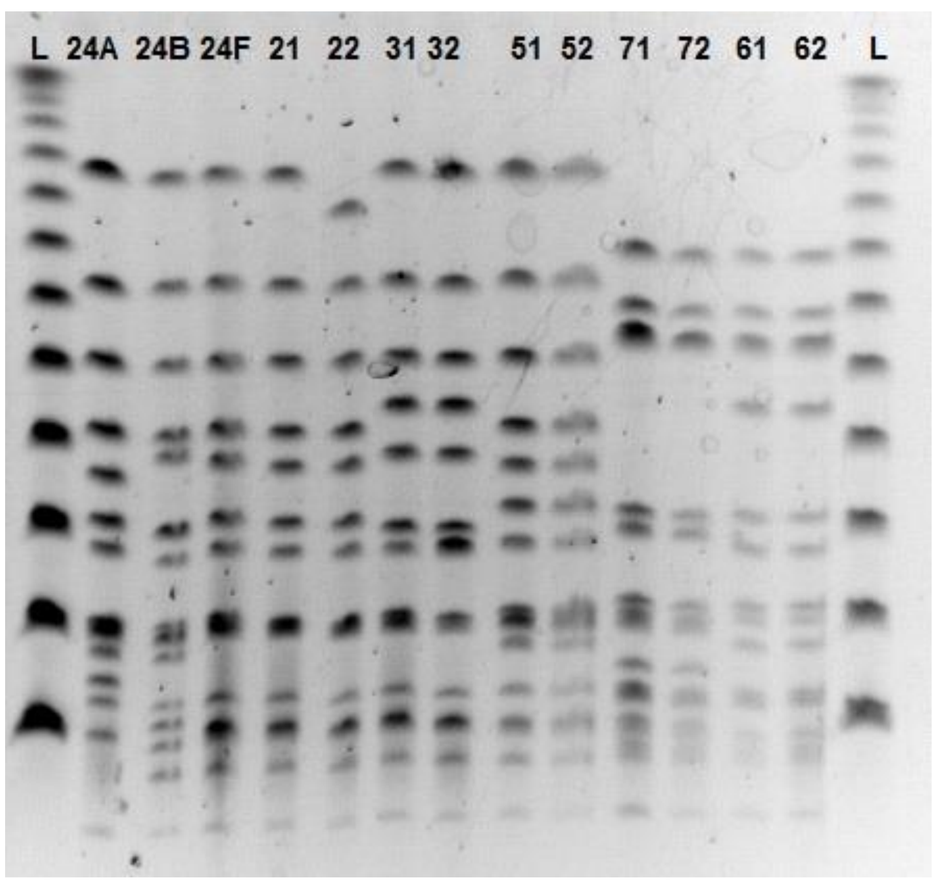

Fig 2: Pulsed Field Gelelectrophoresis of serogroup 24 isolates with unusual factorserum reactions. Of each Isolate a large (1) and a small (2) colony variant were analysed.

Table 2: Multi Locus Sequence Typing of serogroup 24 isolates with unusual factorserum reactions.

Isolate	*aroE*	*gdh*	*gki*	*recP*	*spi*	*xpt*	*ddl*	Sequence Type
2	2	13	2	4	9	4	1	72
3	2	13	2	4	9	4	1	72
5	2	13	2	4	9	4	1	72
6	7	11	10	1	6	8	14	162
7	7	11	10	1	6	8	14	162
8	7	11	10	1	6	8	14	162

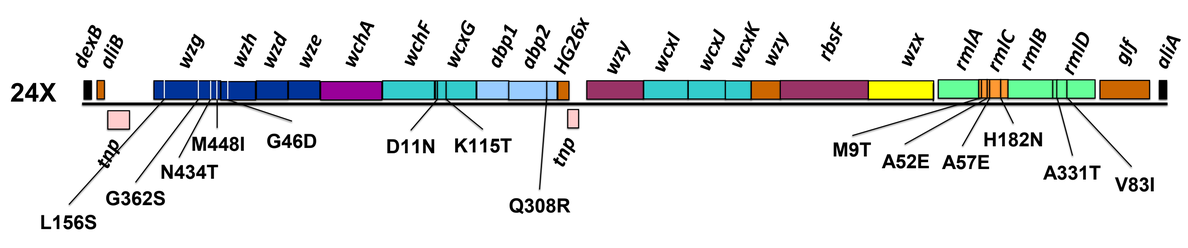

Fig 3: Genetic organization of the putative new serotype 24X. Amino acid exchanges that could have relevance are indicated.

Mutations leading to amino acid exchanges which might be relevant for gene function were found in the *wzg* and *wzh* genes, the *wcxG* gene, the *abp2* gene and the *rmlC*, *rmlB* and *rmlD* genes (**Fig. 3**).

The rhamnose genes look like those of serotype 24F and do not contain the frameshifts found in serotype 24B.

The *rmlC* gene of the serotype 24X isolates contains four possibly relevant mutations.

CONCLUSIONS

- Six isolates showed a postive reaction with groupserum 24, but factorserum reactions were inconclusive.
- The aberrant isolates showed positive reactions with both factorserum 24d (specific for 24F) as well as factorserum 24e (specific for 24B).
- The genomes of the six serogroup 24 isolates are clearly different from extant serogroup 24 serotypes, and also differ from each other as concluded from PFGE.
- The isolates showed STs common to serogroup 24 isolates: ST 72 and ST 162.
- The capsular cassettes of the isolates are identical within each sequence Type group and show highest similarity to those of serotypes 24F and 24B.
- Between the two sequence type groups (ST72 and ST162) there are only four amino acid differences.
- As in seroytpes 24F and 24B, the wzy and rbsF genes are present in the isolates.
- Possibly relevant amino acid exchanges were found in the the *wzg*, *wzh*, *wcxG*, *abp2, rmlC*, *rmlB* and *rmlD* genes
- These isolates may represent a novel serotype within serogroup 24.