Identical novel multidrug resistant Integrative Conjugative Element found in *Haemophilus influenzae* strains of different sequence types from the same geographical region of Norway

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P0776

INTRODUCTION & PURPOSE

Haemophilus influenzae colonises respiratory tract in humans and causes both invasive and non-invasive infections. Resistance to extended-spectrum cephalosporins in *H. influenzae* is rare in Europe. Horizontal transfer of resistance genes in *H. influenzae* is often facilitated by Integrative Conjugative Elements (ICEs).[1] ICEs are self-transmissible mobile genetic elements that encode apparatus for their own excision from the donor chromosome, subsequent circularization, conjugation and reintegration into the recipient chromosome.[2] In this study we defined the acquired resistance gene locus of *H. influenzae* strains showing an extensive multidrug resistant (MDR) pattern, including resistance to extended–spectrum cephalosporins due to different penicillin-binding protein 3 (PBP3).[3]

MATERIALS & METHODS

Two clinical non-invasive strains of extensively MDR and non-typeable *H. influenzae* (F and G, Table 1) isolated in March/April 2013 from patients living in Hordaland County, Norway, were whole-genome sequenced (WGS) by Illumina MiSeq. The strains belonged to the same clonal complex and had related sequence types (six of seven alleles shared) by multi-locus sequence typing (MLST). Acquired resistance genes were identified using ResFinder.[4] ICE structure was found by Basic Local Alignment Search Tool (blast.ncbi.nlm.nih.gov), subsequent mapping to ICE*Hin1056* sequence, and then contig gap closure with PCR and Sanger sequencing. Pairwise comparisons were displayed by Artemis comparison tool[5] using files generated by WebACT (www.webact.org/) (Figure 1).

TABLE 1. Isolates and their characteristics.

Strain or isolate	MLST clonal complex (CC)	MLST sequence type (ST)	PBP3 group[a]	Phenotypic beta-lactam resistance[b]	Phenotypic non-beta-lactam resistance[c]	Acquired resistance genes[d]	Patient category[e]
F	CC-ST503	ST1282	III-like+	B,Ai,Cf,Ct,Cx,Ci,Cp,Co	C,T,Ts	bla_{TEM1}, *catA*, *tetB*	GP
G	CC-ST503	ST159	III+	B,Ai,Cf,Ct,Cx,Cp,Co	Q,C,T,Ts	bla_{TEM1}, *catA*, *tetB*	OC
G2	CC-ST503	ST159	III+	B,Ai,Cf,Ct,Cx,Cp,Co	Q,C,T,Ts	To be determined	H
G3	CC-ST503	ST159	III+	B,Ai,Cf,Ct,Cx,Cp,Co	Q,C,T,Ts	To be determined	OC

[a] Classification based on PBP3 substitutions. III+: S385T, L389F, N526K; III-like+: S385T, L389F, R517H [b] B, beta-lactamase (*bla*); Ai, aminopenicillin-beta-lactamase inhibitor combinations; Cf, cefuroxime; Ct, cefotaxime; Cx, ceftriaxone; Ci, cefixime; Cp, cefepime; Co, ceftaroline [c] C, chloramphenicol; T, tetracycline; Ts, trimethoprim-sulfamethoxazole; Q, quinolone [d] *bla*, beta-lactamase; *catA*, chloramphenicol resistance gene; *tetB*, tetracycline resistance gene [e] GP, general practice; OC, outpatient clinic; H, hospitalised

RESULTS

Strains F and G harboured identical acquired resistance genes towards penicillins, chloramphenicol and tetracycline, but on different WGS contigs. The same resistance pattern is displayed by the ICE*Hin1056* found in *H. influenzae* strain 1056 from UK. Pairwise BLAST revealed presence of large fragments of the 59,4-kb ICE*Hin1056* in both strains.[1] Gap closures revealed a 100% identical putative novel 64,7-kb ICE termed ICE*HinF* and ICE*HinG* in the two respective strains (Figure 1). A circular from of this ICE (Figure 2) was demonstrated by PCR using primers directed outwards from the ends. We currently investigate two other strain G isolates from the same geographic region (G2 and G3, Table 1) with similar extensive MDR pattern for presence of this ICE. Conjugation studies to reveal transferability of this ICE will also be performed.

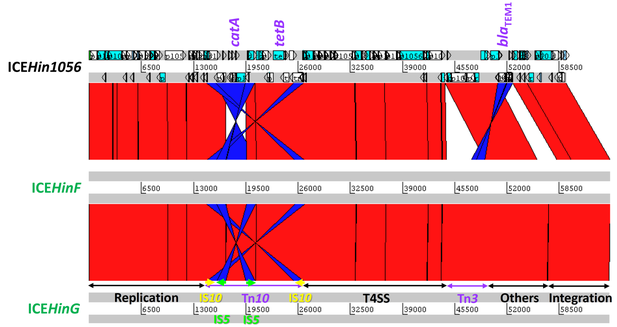

FIGURE 1. Pairwise comparisons of the novel ICEs and ICE*Hin1056*. The red and blue bands represent the forward and reverse matches, respectively.

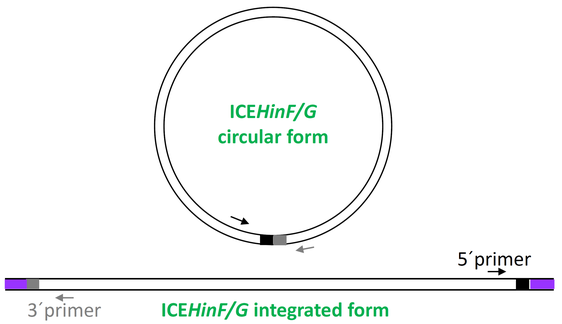

FIGURE 2. Schematic drawing of integrated and circular forms of ICE*HinfF/G*. Left and right ICE ends are colored gray and black. Native chromosomal DNA is shown in purple.

CONCLUSIONS

Two extensively MDR *H. influenzae* strains from the same geographical region, with related MLST profiles but different PBP3 resistance genotypes and quinolone susceptibility profiles, share an identical novel MDR ICE. Demonstration of ICE circular forms indicates that the enzymes involved in excision of this ICE are functional.

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