Typing of *Fusobacterium necrophorum* Strains Using Polymerase Chain Reaction (PCR) Based Methods

Ahmad Rahmati1*, Jon S. Brazier2

1Department of Basic Sciences, Faculty of Medical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2Anaerobic Reference Laboratory, NPHS Microbiology, Cardiff University Hospital of Wales, UK

*Corresponding author: Ahmad Rahmati, Department of Basic Sciences, Faculty of Medical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran. Tel: +98 9143135867. E-mail: rahmatia@iaut.ac.ir

Submitted: November 10, 2013; Revised: January 6, 2014; Accepted: July 7, 2014

**Background**: *Fusobacterium necrophorum* as a non-spore-forming Gram-negative anaerobic bacillus is an important human and animal pathogen. It may cause severe systemic infections (Lemierre's syndrome) and some other infections. The aim of this study was to subtype *Fusobacterium necrophorum* by using PCR methods.

**Materials and Methods**: Twenty five strains of *Fusobacterium necrophorum* subspecies *funduliformis* were used. Extraction of DNA and typing of the strains using REP-PCR, ERIC-PCR and BOX-PCR were done.

**Results**: Molecular typing of *Fusobacterium necrophorum* using REP1-R1 and REP2-I primers generated 2 to 5 amplicons ranging in size from 1500bp to 2000bp. GelCompar comparison of banding patterns revealed seven distinct ribotype strains from 23 strains tested of which most were 2 and 4 with 8 and 7 strains respectively. BOX-PCR subtyping generated 2 to 7 comparable amplicons ranging in size from approximately 600bp to more than 2000bp. ERIC-PCR subtyping generated 6 to 11 amplicons ranging in size from approximately 100bp to 1500bp.

**Conclusion**: *F. necrophorum* strains have genomic variations that suggest they are never truly clonal in nature, or they may have undergone localized genetic variation across worldwide. This study also showed subtypes existing in *Fusobacterium necrophorum* species. We have demonstrated that *Fusobacterium necrophorum* REP-PCR types can be divided into seven, three subtypes by BOX-PCR and six subtypes by ERIC-PCR. BOX-PCR typing proved to be the most discriminatory method, yielding two-seven major bands. The sample size was too small to interpret statistically.

**Keywords**: Typing, *Fusobacterium necrophorum*, PCR

1. **Background**

*Fusobacterium necrophorum* is a non-spore-forming, Gram-negative anaerobic bacillus that may exist as part of the human normal microbial flora. It has been divided into two sub-species called *F. necrophorum* ss. *necrophorum* (biovar A) and *F. necrophorum* ss. *funduliformis* (biovar B). It is an important human and animal pathogen. It may be the causative agent of localized (persistent sore throat syndrome) (1), orbital cellulitis or severe systemic infections. Systemic infections due to *F. necrophorum* are known as Lemierre’s syndrome, which is characterized by acute jugular vein septic thrombophlebitis that progresses to sepsis (2); postanginal sepsis or necrobacillosis (3, 4). Septic polyarthritis is rarely caused in teens-agers following infection with *F. necrophorum* (5). Origin of the infection is unknown but there are evidences that the infection originates from human or animal source (6, 7). This bacterium has a role in upper body infections such as mediastinitis, otitis media, mastoiditis and sinusitis (7). Metastatic abscesses in lungs, liver, kidneys and pyogenic arthritis/osteomyelitis (8, 9), endocarditis (10, 11) and rarely pneumonia and jaundice (12). Untreated or improperly treated cases can be fatal. The most common course of severe infections in humans is a progressive illness from tonsillitis to septicemia in previously healthy young adults, which progresses to fever with rigors and leads to septic shock. It also causes infections in animals: calf diphtheria, labial necrosis in lambs, liver abscess in cattle, foot rot in ungulates, lumpy jaw and necrotic abscesses in wallabies, most of these infections are fatal. *F. necrophorum* possesses important virulence factors such as endotoxin (LPS), leukotoxin, haemolysin and haemagglutinin, which enable it to cause variety of diseases (2).

2. **Objectives**

The aim of this study was to subtype *Fusobacterium necrophorum* by using PCR methods.

3. **Materials and Methods**

3.1. **Bacterial strains**

Twenty five strains of *Fusobacterium necrophorum* subspecies *funduliformis* were obtained from the Anaerobic Reference Laboratory, NPHS Microbiology, Cardiff, University Hospital of Wales, UK, which had been isolated from patients with septicaemia, tonsillitis and pleuritis.

3.2. **DNA extraction and PCR assays**

Extraction of DNA and typing of the strains using PCR were done by the method as described previously (13) with some modifications. Briefly, DNA of the strains was extracted using Chelex-100 (Bio Rad, Hemel Hempstead, UK) and was suspended in 2mL of High Performance Liquid Chrom atography-grade water (HPLC). It was then vortexed and dispensed in 100µL aliquots in 0.5mL tubes. One micro liter loopful of cells was added to the suspension, heated in a gently boiling water bath for 12 min and centrifuged for 10min at 15000g. The supernatant (40µL) was used as a crude DNA template.

3.3. **REP- BOX- and ERIC PCR assays**

For Repetitive Extragenic Palindromic Elements-PCR (REP-PCR), one microliter of extracted genomic DNA was used per reaction. Each 25µL of PCR mixture comprised 20µL of mastermix (including PCR buffer, deoxynucleoside triphosphate, MgCl2, primers of REP1-R-I: 5'-III CGICGI CATCIGGC-3' and REP2-I5'-IICCITTTACIGGGCCTAC-3').
In this method of PCR-subtyping, the strains were divided into six groups and the most of the strains were in group 2 (Fig. 3).

5. Discussion

_Fusobacterium necrophorum_ as an important human and animal pathogen and causative agent of localized (1), orbital cellulitis or severe systemic infections, is identified by using conventional methods. Although using DNA sequencing methods are widely used to type and identify bacterial species in many clinical microbiology laboratories (1, 3, 4), however, subtyping of the bacteria by these methods is needed to establish relationship among the strains isolated from different regions and sources for epidemiological purposes. In this study, there was no meaningful relationship between source of the organism and different subtypes.

_Fusobacterium necrophorum_ strains have genomic variations that suggest they are never truly clonal in nature, or may have undergone genetic variation worldwide (14). Evidence to support the existence of subtypes of the other species of _Fusobacterium nucleatum_ ( _F. nucleatum_ ) has been reported previously (15). This study also showed existence of subtypes in _Fusobacterium necrophorum_ species.
We have demonstrated that *Fusobacterium necrophorum* REP-PCR subtypes can be divided into total seven groups, three subtypes by BOX-PCR and six subtypes by ERIC-PCR. From these PCR methods REP-PCR was not suitable for subtyping the strains. Three subtypes from REP and ERIC-PCR were different from other subtypes in having extra bands of 2000bp and 800bp in REP and ERIC-PCR, respectively.

The results obtained from different studies suggest that although BOX-PCR, REP-PCR or ERIC-PCR typing has shown to be sensitive, quick and convenient for the differentiation of some bacterial strains such as *Xanthomonas, Pseudomonas* and *E. coli* it does not appear to be as effective for *Fusobacterium necrophorum*. REP-PCR also lacked the power to discriminate between isolates of *F. necrophorum*. Although this method has the capability to differentiate other bacteria (16-18). From these methods, BOX-PCR typing proved to be the most effective method, yielding two-seven major bands.

6. Conclusion

Twenty five strains of *Fusobacterium necrophorum* were isolated from different patients were analyzed by three PCR-based typing methods in order to determine genomic diversity within the strains. The three methods used were REP-PCR, BOX-PCR, and ERIC-PCR. The performance of each typing method was assessed by comparing the discriminatory power, typeability and reproducibility of each test. All methods had satisfactory levels of typeability and reproducibility, however, BOX-PCR typing was proved to produce the most discrimination, yielding two to seven major bands. *F. necrophorum* strains have genomic variations that propose they are never truly clonal in nature, or they may have undergone genetic variation worldwide.

Conflict of Interests

The authors declare they have no conflict of interests.

Acknowledgements

The authors would like to thank the Ministry of Health, Medical Services and Medical Education of Iran for financial support.

Authors’ Contributions

Ahmad Rahmati performed all the laboratory work and Jon S. Brazier supervised the process.

Funding/Support

This study supported by Ministry of Health, Medical Services and Medical Education of Iran.

References

17. Louws FJ, Fulbright DW, Stephens CT, De Brujin FJ. Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathogens and strains generated with repetitive sequences and PCR. Appl Environ Microbiol. 1994; 60 (7): 2286-95.