# Antimicrobial Susceptibility and Prevalence of Extra-intestinal Enterotoxigenic *Bacteroides fragilis* Among a 5-year Collection of Isolates Causing Sepsis in Kuwait

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## Introduction

- *Bacteroides fragilis* is the most common anaerobe associated with clinical infections. Its susceptibility to various antimicrobial drugs have been reported in different geographic areas, including Kuwait [1,2]. Enterotoxigenic *B. fragilis* (BFT) strains which induce cytopathic effect on the intestinal epithelial cells leading to excessive fluid secretion and tissue damage have emerged as important etiological cause of diarrhea in children and adults. [3]. However, there is evidence that these strains can cross their normal gastrointestinal niches and cause extra-intestinal infections.

## Objective

- To: investigate the antibiotic susceptibility of non-fecal clinical isolates and the prevalence of BFT isolates associated with extra-intestinal infections.

## Materials and Methods

- Isolates of extra-intestinal origin, sent to the Anaerobe Reference Laboratory and identified by VITEK MS (MALDI-TOF System, bioMérieux, Marcy l'etoile, France), were studied.
- Antimicrobial susceptibility testing (AST) was performed with Etest and results interpreted by the recommended criteria of CLSI [4] and FDA 2018 insert for tigecycline.
- Molecular detection of genes encoding enterotoxin (*bft*) production was carried out using *bft*F and *bft*R primers [5].
- Detection of subsets of *bft* genes was by sequencing and correlated to various sepsis. Control strains included in each run were *B. fragilis* R19811 (*bft*-1), *B. fragilis*, ATCC 43858 (*bft*-2) and *B. fragilis* GAI 96462 (*bft*-3).

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## Results

- The patients were aged 1month-95 years (mean, 56.0 years).
- Sources of the isolates were: intra-abdominal infections (IAIs), lower respiratory tract infections (LRTIs), BSIs, wound infections (WIs), and abscesses.

**Table 1:**

- 104 (39.7%) of the 262 isolates studied were *bft*-positive. Their distribution per infective source is shown in this Table.
- Of these 104, 76 (73.1%) were positive for subset genes *bft*-1, 25 (24.0%) *bft*-2 and 3 (2.9%) *bft*-3.
- The majority, 44 (42.3%) and 35 (34%), were associated with intra-abdominal and respiratory tract infections, respectively.

**Table 2:**

- Only metronidazole and tigecycline demonstrated excellent activities against all isolates.

**Table 3:**

- Further analysis showed that isolates positive for subset *bft*-1, *bft*-2 and *bft*-3 were more resistant to clindamycin, metronidazole and piperacillin than *bft*-negative isolates,

**Multidrug-resistant (MDR) isolates:**

- Fifty-four (56.2%) of *bft*-positive isolates were MDR, out of which 46 (85.2%), 7 (13%) and 1 (1.8%) were subsets *bft*-1, *bft*-2 and *bft*-3, respectively.

**Table 1. Infective sources of subsets of *bft*-positive *Bacteroides fragilis***

| Source | Number (%) of subsets of isolates (n=104) | | | Total (%) |
|---|---|---|---|---|
| | *bft*-1 | *bft*-2 | *bft*-3 | |
| Wound infections | 9 (8.7) | 2 (1.9) | 1 (1) | 12 (11.5) |
| Respiratory tract infections | 23 (22.1) | 12 (11.5) | 0 | 35 (34.0%) |
| Blood stream infections | 7 (6.7) | 3 (2.9) | 0 | 10 (9.7%) |
| Abscesses | 2 (1.9) | 1 (1) | 0 | 3 (2.9) |
| Intra-abdominal infections | 35 (33.7) | 7 (6.7) | 2 (1.9) | 44 (42.3) |
| Total (%) | 76 (73.1) | 25 (24.0) | 3 (2.9%) | 104 |

**Table 2.-Antimicrobial susceptibility of all *Bacteroides fragilis* isolates.**

| Antibiotics/ (breakpoint; µg/ml)* | Minimum inhibitory concentrations (MIC: µg/ml) | | | % Resistant |
|---|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ | |
| **Co-amoxiclav (4)** | 0.125 - >256 | 1.5 | >256 | 28 |
| **Clindamycin (2)** | <0.016 - >256 | >256 | >256 | 58 |
| **Imipenem (4)** | 0.023 - >32 | 0.25 | >32 | 12 |
| **Meropenem (4)** | 0.023 - >32 | 0.25 | >32 | 16 |
| **Metronidazole (8)** | 0.023 - >256 | 0.5 | 2 | 4 |
| **Penicillin (0.5)** | 4 - >256 | >256 | >256 | 100 |
| **Piperacillin (16)** | 0.19 - >256 | 96 | >256 | 56 |
| **Cefoxitin (16)** | 0.032 - >256 | 6 | >256 | 26 |
| **Tigecycline (4)**** | 0.094 - 16 | 1 | 4 | 9 |

***CLSI, 2018; **FDA-identified interpretative criteria, 2018**

**Table 3. Antimicrobial resistance pattern of *bft*-positive versus *bft*-negative *Bacteroides fragilis***

| Antibiotics/ (breakpoint; µg/ml) | Number (%) of resistant isolates: *bft*-positive (n=104) | | | | *bft*-negative (n=158) |
|---|---|---|---|---|---|
| | All isolates | *bft*-1 | *bft*-2 | *bft*-3 | |
| **Co-amoxiclav (4)** | 23 (22.1) | 23 (22.1) | 0 (0) | 0 (0) | 40 (26.1) |
| **Clindamycin (2)** | 66 (63.5) | 53 (51) | 12 (11.5) | 1 (1) | 71 (46.4) |
| **Imipenem (4)** | 9 (8.7) | 9 (8.7) | 0 (0) | 0 (0) | 20 (13.1) |
| **Meropenem (4)** | 14 (13.5) | 13 (12.5) | 1 (1) | 0 (0) | 27 (17.64) |
| **Metronidazole (8)** | 5 (4.8) | 4 (3.9) | 1 (1) | 0 (0) | 1 (0.6) |
| **Penicillin (0.5)** | 100 (100) | 78 (75) | 21 (20.1) | 1 (1) | 100 (100) |
| **Piperacillin (16)** | 49 (47.1) | 37 (35.6) | 11 (10.6) | 1 (1) | 36 (22.8) |
| **Cefoxitin (16)** | 16 (15.4) | 15 (14.2) | 1 (1) | 0 (0) | 42 (27.5) |
| **Tigecycline (4)** | 11 (10.6) | 8 (7.7) | 3 (2.9) | 0 (0) | 11 (7) |

## Conclusion

- There was a very high proportion of BFT strains detected among our isolates and overwhelming proportion belonged to the *bft*-1 subsets which were the predominant isolates found in clinical extra-intestinal infections.
- The *bft*-positive isolates were more resistant than the *bft*-negative isolates to clindamycin, metronidazole and piperacillin.

**References**

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