

# ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *Clostridium difficile* ISOLATES FROM FAMILY DAIRY FARMS

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*Clostridium difficile* (CD) is known to be resistant to certain antimicrobials and takes advantage when other bacteria in the gut microbiota decrease in number. Indeed, one of the major risk factors for developing CD infection (CDI) in humans is associated with antimicrobial use. Antimicrobials are also often used on dairy cattle farms and many are similar to those used in human medicine. Most CD studies for antimicrobial susceptibility in cattle were performed on CD isolates from large scale intensive dairy or beef farms, which is the opposite of the most common operating farming model in European Union, family farming. Any antimicrobial resistance of CD isolates in such environment is likely to spread within the family and local community and would have a major impact on the treatment of human community acquired CDI.

A total of 159 CD isolates deriving from 20 family dairy farms (103 from cattle, 56 from the environment) were tested with a customized broth microdilution plate (Micronaut) for 17 antimicrobials that are used in veterinary medicine, in human medicine for treatment of CDI and in epidemiological studies.

All CD isolates were susceptible to antimicrobials that are used for CDI treatment in humans (metronidazole, vancomycin, fusidic acid, tigecycline, linezolid) and to moxifloxacin. Most CD isolates were above the recommended values for erythromycin (93.1%) and daptomycin (69.2%), and 46.5% for clindamycin. High resistance or high MIC values against erythromycin, clindamycin, rifampicin, tetracycline, ceftriaxone and trimethoprim/sulfamethoxazole belonged to CD isolates ribotype 012 (n=5), whereas low resistance against clindamycin, rifampicin, erythromycin and daptomycin belonged to ribotype SLO 060 (n=4).

| ANTIMICROBIAL                 | MICRONAUT-5 CD MIC PLATE - ANTIMICROBIAL CONCENTRATION µg/ml |        |        |        |       |      |     |       |       |       |       |       |       |      |     |      |      |
|-------------------------------|--------------------------------------------------------------|--------|--------|--------|-------|------|-----|-------|-------|-------|-------|-------|-------|------|-----|------|------|
|                               | 70,00195                                                     | 0,0039 | 0,0078 | 0,0625 | 0,125 | 0,25 | 0,5 | 1     | 2     | 4     | 8     | 16    | 32    | 64   | 128 | 256  | >512 |
| Amoxicillin                   |                                                              |        |        | 22,65  | 67,9  | 8,8  | 0   | 0     | 0     | 0     | 0     | 0     | 0,63  |      |     |      |      |
| Ceftriaxone                   |                                                              |        |        |        |       |      |     |       |       |       | 24,52 | 66,04 | 7,55  | 1,26 | 0   | 0    | 0,63 |
| Daptomycin                    |                                                              |        |        |        |       |      |     | 1,88  | 11,32 | 18,24 | 42,15 | 26,41 |       |      |     |      |      |
| Erythromycin                  |                                                              |        |        |        |       |      |     | 1,88  | 5,04  | 78,63 | 10,69 | 110   |       |      | 0   | 0    | 1,88 |
| Fusidic acid                  |                                                              |        |        |        |       |      |     |       | 100   | 0     |       |       |       |      |     |      |      |
| Imipenem                      |                                                              |        |        |        |       |      |     |       |       | 94,97 | 4,4   | 0,53  | 0     |      |     |      |      |
| Clindamycin                   |                                                              |        |        |        |       |      |     | 1,66  | 5,66  | 8,8   | 16,98 | 16,35 | 43,41 | 0,63 | 0   | 2,51 | 0    |
| Linezolid                     |                                                              |        |        |        |       |      |     |       |       | 18,86 | 78,63 | 1,76  | 1,76  | 0    |     |      |      |
| Metronidazole                 |                                                              |        |        |        |       |      |     | 9,82  | 8,18  | 0     | 0     |       |       |      |     |      |      |
| Moxifloxacin                  |                                                              |        |        |        |       |      |     |       |       | 96,24 | 1,88  | 1,88  | 0     |      |     |      |      |
| Oxacillin                     |                                                              |        |        |        |       |      |     |       |       | 98,74 | 1,26  | 0     | 0     | 0    |     |      |      |
| Rifampicin                    |                                                              |        |        |        |       |      |     | 27,67 | 1,88  | 1,26  | 30,2  | 35,85 | 2,51  | 0,53 |     |      |      |
| Tetracycline                  |                                                              |        |        |        |       |      |     |       |       |       |       |       |       |      | 0   | 0,63 | 1,26 |
| Tigecycline                   |                                                              |        |        |        |       |      |     |       |       |       |       | 0,63  | 2,51  |      |     |      |      |
| Trimethoprim/sulfamethoxazole |                                                              |        |        |        |       |      |     |       |       |       |       |       |       |      |     |      |      |
| Vancomycin                    |                                                              |        |        |        |       |      |     |       |       |       |       |       |       |      |     |      |      |

Figure 1: Antimicrobial pattern distribution of minimal inhibitory concentration (MIC) of *Clostridium difficile* isolates. In blue squares - % of CD isolates below the epidemiological cut off; in red squares - % of CD isolates above the epidemiological cut off; vertical line - cut off; red numbers - % of CD isolates with MIC above tested concentrations of antimicrobials. Results were interpreted based on epidemiological cut off values according to EUCAST guidelines.

| COMBINATION OF ANTIMICROBIAL RESISTANCE IN <i>Clostridium difficile</i> ISOLATES FROM FAMILY DAIRY FARMS |            |             |            |              | Total No. (n=159) | %    |
|----------------------------------------------------------------------------------------------------------|------------|-------------|------------|--------------|-------------------|------|
| Erythromycin                                                                                             |            |             |            |              | 148               | 93,1 |
| Erythromycin                                                                                             | Daptomycin |             |            |              | 110               | 68,6 |
| Erythromycin                                                                                             |            | Clindamycin |            |              | 74                | 46,5 |
| Erythromycin                                                                                             |            |             | Rifampicin |              | 9                 | 5,7  |
| Erythromycin                                                                                             |            |             |            | Tetracycline | 5                 | 3,2  |
| Erythromycin                                                                                             | Daptomycin | Clindamycin |            |              | 68                | 42,8 |
| Erythromycin                                                                                             |            | Clindamycin | Rifampicin |              | 7                 | 4,4  |
| Erythromycin                                                                                             |            | Clindamycin |            | Tetracycline | 5                 | 3,2  |
| Erythromycin                                                                                             | Daptomycin |             | Rifampicin |              | 6                 | 3,8  |
| Erythromycin                                                                                             | Daptomycin |             |            | Tetracycline | 2                 | 1,3  |
| Erythromycin                                                                                             | Daptomycin | Clindamycin | Rifampicin |              | 5                 | 3,2  |
| Erythromycin                                                                                             | Daptomycin | Clindamycin |            | Tetracycline | 2                 | 1,3  |
| Erythromycin                                                                                             |            | Clindamycin | Rifampicin | Tetracycline | 4                 | 2,5  |
| Erythromycin                                                                                             | Daptomycin | Clindamycin | Rifampicin | Tetracycline | 2                 | 1,3  |

Figure 2: Combination of antimicrobial resistance in *Clostridium difficile* isolates from family dairy farms. Total No. - total number of CD isolates with the selected combination of antimicrobial resistance

In this study we found that, with the exception of tetracycline and trimethoprim/sulfamethoxazole, the presence of antimicrobial resistance in CD isolates from family dairy farms was against antimicrobials used only in human medicine. Also, multiple-resistance was linked to ribotypes and not to the origin of CD isolates. The low prevalence of these ribotypes makes family dairy farms a possible, but unlikely source of CD with multiple-resistance to antimicrobials.