

The 100 control thymuses showed several patterns of involution with a decrease in number of lymphocytes and relative preservation of the epithelial cells. In four of the 100 cases (age range 53–80 years), epithelial islands and rosette-like formations were seen. In other cases, the architectural arrangement was characterised by anastomosing strands of epithelial elements admixed with thymocytes.

The mean diameter of the epithelial nests was 107 mm (range 41–237 mm).

### Discussion

Approximately 75% of patients with myasthenia gravis have thymic abnormalities, namely lymphoid follicular hyperplasia or thymoma or both.<sup>3,4</sup> Surgical thymectomy results in disease improvement in most myasthenic patients and in non-thymoma cases it is often curative,<sup>5</sup> although the overall prognosis has not been significantly modified in large series of patients who have had thymectomy.<sup>6,7</sup> The surgical approach is useful, however, in preventing spread of the thymoma. In this case we could not demonstrate the presence of a thymoma by computed tomography; nevertheless a thymectomy was performed for therapeutic purposes.

On histological examination, the islands of epithelial cells were interpreted as possible multiple foci of microscopic thymoma. This "microscopic thymoma" had a morphological pattern compatible with the descriptions of Rosai and Levine.<sup>1</sup> Moreover, Pescarmona *et al* reported three other cases showing microscopic epithelial lesions (0.2–0.4 mm in diameter) consistent with foci of thymoma on the basis of their morphological appearance.

Since it is also possible to find epithelial clusters in involuted thymuses,<sup>8</sup> we analysed as controls 100 consecutive thymuses with differ-

ent degrees of involution obtained at necropsy. In four of these 100 cases, epithelial islands and very small rosette-like formations were observed. These microscopic changes were similar to those present in microscopic thymoma, especially the rosette-like formations. The findings of Rosai and Levine were similar, as were those of Pescarmona *et al*,<sup>2</sup> and from a morphological point of view it is quite impossible to distinguish between the two conditions. Pescarmona suggested a possible multifocal origin of thymoma. Our case supports this suggestion and underlines the association between myasthenia gravis and multifocal thymoma not associated with lymphoid follicular hyperplasia of the thymus.

In conclusion, in order to establish the unequivocal presence of a thymoma in patients suffering from myasthenia gravis it is necessary to obtain histological samples of the entire gland, particularly in those cases without macroscopically evident lesions. Finally, it is important to realise that computed tomography of the chest is unable to detect microscopic sized thymomas.

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## Evaluation of the API-Campy System in the biochemical identification of hippurate negative campylobacter strains isolated from faeces

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### Abstract

The aim was to evaluate the efficacy of the API-Campy system in the biochemical identification of 62 hippurate negative campylobacter strains isolated from the faeces. The strains were identified manually as 34 nalidixic acid susceptible *C coli* (NAS), 20 nalidixic acid resistant *C coli* (NAR), and eight *C lari*. The 34 strains of

NAS *C coli* were identified as such by the API-Campy system. Of the 20 strains of NAR *C coli*, 15 (75%) were correctly identified by the commercial system. None of the five NAR *C coli* strains which were also erythromycin resistant was identified as such by the system. The eight *C lari* strains could not be identified by the API-Campy system because the bionumber obtained

**does not exist in the database of the computer system. The API-Campy system could be very useful for the identification of NAS *C coli*. However, failure to allow for a higher percentage of resistance to nalidixic acid in this species does not permit good identification of NAR strains. More important discrepancies are observed in *C lari* strains. In order to improve the identification of NAR *C coli* and *C lari* strains, it is advisable to include, or recommend as complementary, the indoxyl acetate hydrolysis test.**

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The genus *Campylobacter* is now the second most important group in the aetiology of diarrhoea in our country, affecting the infant population preferentially. Included in this bacterial group are species capable of growing at 42°C, the most important from a clinical point of view being *C jejuni*, *C coli*, *C lari*, and, more recently, *C upsaliensis*.<sup>1</sup>

The observation of Harvey<sup>2</sup> that the hippurate hydrolysis test allows clear differentiation between the species *C jejuni* and *C coli* simplifies the biochemical identification of these species. However, the routine use of this test alone for the identification of strains isolated in faeces may lead to certain taxonomic errors.<sup>3</sup> In addition to the use of this differential test, most workers recommend the nalidixic acid susceptibility test (30 µg discs) to complete the identification of strains.<sup>13</sup> However, the progressive increase in the rate of nalidixic acid resistance in *C jejuni* strains (nearly 40%), and more so in *C coli* (30-50%), causes difficulty in distinguishing between nalidixic acid resistant *C coli* strains and *C lari*.<sup>45</sup> The introduction of additional biochemical tests, such as indoxyl acetate hydrolysis, growth in anaerobiosis in the presence of 1% trimethylamine-*N*-oxide hydrochloride (TMAO), or rapid H<sub>2</sub>S production, is necessary.<sup>13</sup>

The development of a semiautomated commercial system would facilitate the identification of the different strains. The aim of this study was to evaluate the efficacy of the commercial API-Campy system (bioMerieux, France) in the biochemical identification of 62 hippurate negative campylobacter strains isolated in the faeces of patients with diarrhoea.

Only those strains which were repeatedly hippurate negative in the hippurate hydrolysis test<sup>6</sup> were included in the study. The 62 strains studied were isolated in routine stool cultures processed for the isolation of the different enteropathogens. For the isolation of *Campylobacter* spp we used the Campylosel agar (bioMerieux, France) incubated at 42°C for 48 hours in a microaerophilic atmosphere. All colonies growing in this medium were subjected to the oxidase, catalase, motility, and Gram stain tests. Species identification was carried out by means of the following tests: hippurate and indoxyl-acetate hydrolysis, H<sub>2</sub>S production in TSI and in the rapid test, nitrate reduction,

growth in 0.1% TMAO, 1% glycine, and at 25°, 37°, and 42°C, and susceptibility to nalidixic acid (30 µg) and cephalothin (30 µg).<sup>13</sup>

The strains were identified at 34 (54.8%) nalidixic acid susceptible *C coli* (NAS), 20 (32.2%) nalidixic acid resistance *C coli* (NAR), and eight (13%) *C lari*. All the strains were replated on blood agar with 5% sheep's blood and maintained for later use. The study also included the strains *C coli* ATCC 33559, and *C lari* ATCC 35221.

All strains were subjected to biochemical identification by the API-Campy system following manufacturer's instructions. The interpretation of the results was carried out by means of the computerised automatic API-Campy analytical profile index (API index V 1.0) (bioMerieux).

The 34 strains of NAS *C coli* were identified as such by the API-Campy system. Of these, 30 (88.2%) presented the bionumber 6421504, with variations in only four strains (11.8%) due to their resistance to erythromycin (bionumber 6421506). In addition, in two strains (5.8%) this resistance was associated with a positive citrate assimilation test (bionumber 6401572). In all these cases the strains were identified with a probability of 98.7% and 99.9%, with a T index (or the average) of 0.90, 0.84, and 0.83 respectively. The type strain *C coli* ATCC 33559 was correctly identified with the bionumber 6421504 because of its susceptibility to erythromycin.

Of the 20 strains of NAR *C coli*, 15 (75%) were correctly identified by the API-Campy system (bionumber 6421704) although with a probability of correct identification of only 75.7% and a T index of 0.74. According to the API index these strains should be distinguished from the species *C fetus*, since the biochemical database of the computer system contains the information that this species presents 81% resistance to nalidixic acid, against 10% for the species *C coli*. We consider that these percentages are not true for *C coli* or, if they were in the past, they are no longer, since there have been many studies showing an overall increase in resistance to nalidixic acid and to the rest of the fluoroquinolones in the majority of thermotolerant campylobacter, and specially in *C coli*.<sup>7,8</sup> The nalidixic acid resistance test can no longer be used in our country to distinguish *C coli* from *C lari*, and it is now necessary to use a new biochemical identification system in which the indoxyl acetate hydrolysis test appears to be essential.<sup>8</sup>

On the other hand, none of the five NAR *C coli* strains (25%) which were, in addition, erythromycin resistant, was identified as such by the API-Campy system. In all five the bionumber 6421706 was obtained. This number does not exist in the API index database, in spite of the fact that erythromycin resistance is considered in strains analysed. These five strains were identified manually as NAR *C coli* in view of their hippurate negativity and indoxyl acetate positivity. It seems, therefore, that the database of the system does not allow for the possibility that the species *C coli* is resistant to these two antibiotics simultaneously, a phe-

nomenon which is present, at least in our strains, in 12.5% of nalidixic acid resistant strains and 3.7% of *C coli* strains.

The eight strains previously identified at *C lari* could not be identified by the Api-Campy system. For all of these the bionumber 6401704, which does not exist in the database of the API index, was obtained. This is apparently due to the fact that in the database the species *C lari* is given only 3% resistance to nalidixic acid when in fact one of the essential requirements for the identification of this species is resistance to this antibiotic, an attribute that was used by Benjamin *et al*<sup>9</sup> to classify this species as belonging to the NARTC group.

The *C lari* type strain ATCC 35221 gave the bionumber 2020204 with an identification of 72.8% and a T of 0.75, since its behaviour was nalidixic acid resistant. The API index recommends the use of the tests of growth at 25°C in 1% glycine and in 1.5% NaCl, and resistance to cephalothin, for the differentiation of *C lari* from two other closely related species, *C fetus venereal* and *C fetus fetus*. In our series all strains were positive in the esterase, reduction of triphenyl tetrazolium chloride, alkaline phosphatase, assimilation of succinate, and resistance to nalidixic acid and cefazoline tests, and negative in the l-arginine arylamidase test.

The API-Campy system does not specify the enzymatic substrate used in the esterase activity test, although it gives 5% positivity for *C lari*. While it is true that no *C lari* strain tolerates the presence of 1% triphenyltetrazolium chloride (TTC), it seems that some strains are able to reduce it, although only weakly.<sup>9</sup> Since the tests which were positive in our *C lari* strains are considered positive by the API index at percentages of around 5–18%, this possibility should be included with the bionumber which takes it into account, that is 6421704. However, this bionumber corresponds with the species *C coli*. Only the tests for esterase, alkaline phosphatase, and succinate assimilation permit a reasonably clear differentiation between these two species.

In view of these results it would seem that the API-Campy system could be very useful for the identification of NAS *C coli* strains. However, the failure to allow for higher percentage of resistance to nalidixic acid in this species does not allow good identification in NAR strains. More important discrepancies are observed in *C lari* strains in the different biochemical tests and particularly in the low percentage of nalidixic acid resistance to this species. We consider that, in order to improve the identification of NAR *C coli* strains and *C lari*, it is advisable to include, or to recommend as complementary, the indoxyl acetate hydrolysis test.<sup>10</sup> It therefore seems necessary to introduce certain modifications in the database of the API index V1.0 system to achieve a higher degree of efficacy in the identification of thermotolerant campylobacter strains isolated in patients with diarrhoea.

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