The need to verify of positive Mycoplasma hominis results obtained using the Mycoplasma IST2 tests

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Konieczność potwierdzania dodatnich wyników badań w kierunku Mycoplasma hominis uzyskanych testem Mycoplasma IST 2

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Summary

The study draws attention to the possibility of falsely positive and negative results obtained by commercial test for detection of Mycoplasma hominis in clinical samples. Material/Methods: 5,715 samples were compared using the Mycoplasma IST 2 kit (BioMérieux) with conventional culture on PPLO (pleuropneumonia like organism) media. The discordant results were verified by PCR method using primers specific for 16S rRNA for M. hominis. Result: Due to bacterial and/or fungal flora overgrowth in 190 samples, the results for M. hominis were impossible to read and these specimens were excluded for analysis. The correlation of the two methods showed compliance for 5341 out of 5525 samples analyzed. Of 240 discordant results, (positive by the the Mycoplasma IST 2 kit) only 26.6% of these samples the presence of M. hominis was confirmed by growing on PPLO media and PCR assay. The sensitivity and specificity of the Mycoplasma IST 2 test as compared to the culture method as a “gold standard” were determined as 91% and 96%, respectively, while the positive and negative predictive value of the commercial test was 27% and 99%, respectively. Conclusions: In view of positive results obtained by the Mycoplasma IST 2 kit for M.hominis should be verified by other diagnostic methods.

Streszczenie

Praca zwraca uwagę na możliwość uzyskania fałszywie dodatnich i fałszywie ujemnych wyników badań w kierunku Mycoplasma hominis uzyskanych w kierunku Mycoplasma hominis testem Mycoplasma IST 2

Materiał i Metody: 5715 próbek porównano przy użyciu testów Mycoplasma IST 2 (BioMérieux) z hodowlą na podłożach PPLO. Niegodne wyniki zostały zweryfikowane metodą PCR przy zastosowaniu starterów specyficznych dla rRNA 16S dla M. hominis. Wyniki: Ze względu na obecną w materiale klinicznym bakteryjną i / lub grzybiczą florę, wyniki w kierunku M. hominis były niemożliwe do odczytania w 190 próbkach i zostały one wyłączone z analizy. Porównanie dwóch metod wykazało zgodność wyników dla 5341 z 5525 analizowanych próbek. Z 240 niezgodnych wyników (dodatnie w teście Mycoplasma IST 2), tylko w 26,6% próbkach obecność M. hominis potwierdzono hodowlą na podłożach PPLO (pleuropneumonia like organism) i metodą PCR. Czułość i swoistość testów Mycoplasma IST 2 względem metody klasyfikacyjnej hodowlą, jako „złotego standardu”, określono na 91% i 96%, natomiast dodatnią i ujemną wartość predykcyjną tych testów określono odpowiednio na 27% oraz 99%. Wnioski: dodatnie wyniki w kierunku M. hominis uzyskane testem Mycoplasma IST 2 należy weryfikować innymi metodami diagnostycznymi.

Key words: culture Mycoplasma hominis, Mycoplasma IST 2, PCR

Słowa kluczowe: hodowla Mycoplasma hominis, Mycoplasma IST 2, PCR

Introduction

Microorganisms from the Mycoplasmataceae family are characterized by unique properties, such as a very small size of both their cells (with the diameter of 0.3 μm) and colonies (between 100 and 500 μm), simplified metabolism, ability of intracellular existence, absence of cell wall, slow growth...
Material and methods

In the years 2008-2010, a total of 5,715 samples were tested for the presence of mycoplasmas and ureaplasmas. The specimens were collected from the genitourinary tract of adult patients and from the respiratory tract of newborns. Each sample was inoculated on a the Mycoplasma IST 2 commercially available test, and classic cultures results were not in agreement. In 178 cases, no M. hominis growth was observed on solid media both after the first and second passage (PPLO broth followed the growth on solid medium). These discordant results were compared and verified with PCR method. The results are shown in Table 1. In 62 (25.8%) of 240 positive samples by the Mycoplasma IST2 kit, M. hominis was also confirmed by PCR. In two cases the presence of M. hominis was confirmed by PCR only. Of the 5285 negative results obtained by the Mycoplasma IST2 tests 6 of M. hominis PPLO cultures were reported as positive.

Results

Of the 5,715 investigated samples, in 190 cases the presence of abundant bacterial and/or fungal flora was observed. Bacterial overgrowth did not allow for correct reading and interpretation of results. These samples were excluded for further analysis.

M. hominis was detected in 240 (4.3%) samples according to positive results by the Mycoplasma IST 2 kit. However using PPLO media M. hominis was confirmed only in 68 (1.2%) analyzed samples. The results obtained using the commercially available test, and classic cultures results were not in agreement. In 178 cases, no M. hominis growth was observed on solid media both after the first and second passage (PPLO broth followed the growth on solid medium). These discordant results were compared and verified with PCR method. The results are shown in Table 1. In 62 (25.8%) of 240 positive samples by the Mycoplasma IST2 kit, M. hominis was isolated on solid and liquid PPLO media and the presence of M. hominis was also confirmed by PCR. In two cases the presence of M. hominis was confirmed by PCR only. Of the 5285 negative results obtained by the Mycoplasma IST 2 test 6 of M. hominis PPLO cultures were reported as positive.
The titer of *M. hominis* was above 10^4 CFU/ml (colony forming units) in all 64 positive samples obtained by the Mycoplasma IST 2 kit and confirmed by PCR. Moreover all these strains were resistant to erythromycin according to Mycoplasma IST 2 test. Based on literature and also our previously observations *M. hominis* strains are naturally resistant to erythromycin [5, 6]. Taking into account this property we decided to examine all discordant samples (n=176) for sensitivity to erythromycin results in parallel with the titer of *M. hominis* obtained by the Mycoplasma IST 2 kit. Results are shown in Table 2.

In this group of samples, no correlation was noted between the presence of *M. hominis* and the expected resistance to erythromycin. Only in 21.6% of cases did Mycoplasma IST 2 test demonstrate resistance to erythromycin, while the majority of samples (78.4%) showed a moderate or complete sensitivity to the macrolide.

While analyzing these 176 samples where Mycoplasma IST 2 test suggested *M. hominis* presence, but results obtained employing other methods failed to confirm the presence of this bacteria, no association was observed between the semi-quantitative results (a titer above 10^4 CFU/ml or below 10^4 CFU/ml) in BioMérieux test. In only 29 cases (16.5%) only, the test indicated a titer above 10^4 CFU/ml.

The correlation between the results obtained by the classic PPLO cultures and the Mycoplasma IST 2 kit was determined as 96%. The sensitivity and specificity of the Mycoplasma IST 2 test as compared to the “golden standard” method was determined as 91% and 96%, respectively, while the positive and negative predictive value of the test was 27% and 99%, respectively.

**Discussion**

After verification of positive results obtained by BioMérieux kit *M. hominis* was reported only in 1.3% cases (70/5525) in our investigation. In the studies conducted by Zdrodowska-Stefanow and co-workers [7, 8] *M. hominis* was isolated in 1% cases in male with urethritis to 3.7% cases in women with urogenital diseases. But in study of Clegg et al. using Mycoplasma IST2 test *M. hominis* was detected in up to 69% of women from Papua-New Guinea [9]. Comparing the results obtained for analyzable by us all samples using the Mycoplasma IST 2 kit and the classic culture on PPLO media, we noted a satisfactory specificity of the Mycoplasma IST 2 test. Clegg at all compared the Mycoplasma IST 2 kit and the classic culture for detection of *M. hominis* in a much smaller group (100 females) and demonstrated lower correlation (91%) of both methods at a slightly higher sensitivity (93%) and lower specificity (87%) of the test as compared to the culture [9].

Our study confirmed falsely positive results in 176 clinical samples and falsely negative in 6 cases tested by the Mycoplasma IST 2. Despite the manufacturer using factors that selectively inhibit growth of fungi and Gram-positive and Gram-negative bacteria other than mycoplasmas, the presence of the former cannot be completely eliminated. One should always take into consideration a possible growth of bacteria that - similarly as ureaplasmas - have the enzyme urease (e.g. *Klebsiella sp.*, *Proteus sp.*), as well as of microorganisms that - just as *M. hominis* - are capable of arginine degradation (such as *Lactobacillus plantarum* or *Pseudomonas aeruginosa*) [10, 11, 12]. A proof of such a possibility can be found in 190 materials, where the presence of other flora demonstrated in cultures on solid media made it impossible to obtain any results of test for the presence of mycoplasmas/ureaplasmas.

Possible falsely positive results obtained using commercially available the BioMérieux test was also suggested by Rastawicki et al. [13]. Evans and coworkers have compared another commercial test (The Mycoplasma Duo kit) with conventional culture using A7 differental agar for the detection of *M. hominis* and ureaplasmas in clinical samples. They obtained discordant results in 4.2 (8/191) specimen but and

**Table I.** Comparison of the results for detection of *M. hominis* obtained in the Mycoplasma IST2 (BioMerieux), culture on PPLO media and PCR.

<table>
<thead>
<tr>
<th>Mycoplasma IST 2 positive results</th>
<th>Mycoplasma IST 2 negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPLO (+)</td>
<td>PPLO (-)</td>
</tr>
<tr>
<td>PCR (+)</td>
<td>PCR (-)</td>
</tr>
<tr>
<td>N=240</td>
<td>N=5285</td>
</tr>
<tr>
<td>62</td>
<td>176</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>5279</td>
<td></td>
</tr>
</tbody>
</table>

*(nt) not tested; (+) positive result; (-) negative result*

**Table II.** Sensitivity to erythromycin of *M. hominis* in comparison with the titer of *M. hominis* in discordant samples (n=176) recognized as positive by using the Mycoplasma IST 2 test.

<table>
<thead>
<tr>
<th>Erythromycin sensitivity</th>
<th>Number samples (%)</th>
<th>Number samples with titer &gt;10^4 CFU/ml (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>38 (21.6)</td>
<td>14 (48.2)</td>
</tr>
<tr>
<td>moderately sensitive</td>
<td>127 (72.1)</td>
<td>11 (37.9)</td>
</tr>
<tr>
<td>Sensitive</td>
<td>11 (6.3)</td>
<td>4 (13.7)</td>
</tr>
</tbody>
</table>

*CFU – colony forming unit*
after verification by PCR method they confirmed falsely positive results only in 2 cases (1%) [14]. In contrast our study falsely positive results were recoded in 3.2% (176/5525) samples tested by the Mycoplasma IST2 kit. Since all strains of \textit{M. hominis} are resistant to erythromycin, the individual user of the Mycoplasma IST 2 test should pay attention to the results of drug resistance [15, 16]. Lack of resistance or demonstrating moderate sensitivity to erythromycin should arouse suspicion of a false result. As it follows from long-term experience of the authors, when isolating ureaplasmas, especially with the titer above $10^4$ CFU/ml, the Mycoplasma IST 2 test may also show positivity for \textit{M. hominis}. This may be possibly associated with transfer of urea-arginine broth with ureaplasmas into the wells which are used for the detection of \textit{M. hominis} and drug resistance. Proposing such a conclusion is warranted by the fact that in all the 176 cases suspected of yielding falsely positive results for \textit{M. hominis}, we detected ureaplasmas, with titers above $10^4$ CFU/ml. Also Kechagia et al., in samples containing mixed \textit{M. hominis} and \textit{U. urealyticum} observed a higher incidence of intermediate susceptibility strains to macrolides [17].

The level of positive and negative predictive value of the Mycoplasma IST2 kit indicate that although a negative result obtained with the use of this test may be considered reliable however the samples positive for \textit{M. hominis} positivity should be absolutely confirmed by another method, e.g. cultures on PPLO media or PCR.

In a small number of diagnostic laboratories, clinical material is inoculated both into commercial diagnostic strips and solid media for mycoplasmas and ureaplasmas detection. This simultaneously inoculations would be greatly contributed to decreasing the number of falsely positive results in tests for \textit{M. hominis}. Parallel inoculation into solid medium would also allow for determining whether other bacterial flora is present in the investigated sample that might result in false interpretation of the commercial test result. Although the use of several methods at the same time increases the cost but their use is necessary for the correct interpretation of test results for \textit{M. hominis}.

Conclusions

In view of possible falsely positive and negative results obtained while testing for \textit{M. hominis} by the Mycoplasma IST 2 kit, it would be justified to verify especially positive results by another diagnostic method.

References