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BIOTECNOLOGIA
MEDICINAL

I IBERIAN CONGRESS ON
MEDICINAL
BIOTECHNOLOGY

BOOK OF ABSTRACTS

ENCONTRO DE BIOTECNOLOGIA MEDICINAL

18 DE MAIO DE 2018

ESCOLA SUPERIOR DE SAÚDE
POLITÉCNICO DO PORTO

IBERIAN CONGRESS ON MEDICINAL BIOTECHNOLOGY

MAY 18TH, 2018

SCHOOL OF HEALTH
POLYTECHNIC OF PORTO

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ISBN: 978-989-20-8533-3

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Screening of positive urines using Sysmex UF-1000i flow cytometer and identification by MALDI-TOF mass spectrometer

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Introduction: Urinary tract infections (UTIs) are the most common infections both in hospitalized and community patients often leading to severe complications. Gram-negative bacilli are the most prevalent microorganisms often resistant to main antimicrobial agents. Microbiology diagnosis is necessary but take at least 48 hours to provide results. Sysmex UF-1000i is an automated urine flow cytometer that have been described as able to avoid the culture on negative samples. We evaluated this equipment in order to screen positive urines with gram-negative bacilli >1 x 105/mL comparing it with the routine method. Additionally, we have developed an extraction protocol for identification of the strains directly from urine on MALDI-TOF.

Material and methods: One-hundred thirty-nine urine samples from routine microbiology laboratory were analyzed on Sysmex UF-1000i. Those showing values > 1 x 105/mL rods were centrifuged according a developed protocol and analyzed on MALDI-TOF (4 spot for each sample) from Bruker. All the results were compared with routine analysis: semi-quantitative smear in CLED agar (BioMérieux), overnight incubation at 37ºC and identification from the colonies by MALDI-TOF. The quantification of microorganisms was recorded and compared to CFU determination using a paired samples t-test ($p < 0.05$). A proportion of agreement (PA) was determined between MALDI-TOF performed directly from urine samples and from colonies. When Sysmex UF-1000i detected cocci/mixed population or negative (<1 x 104/mL) no further studies were done.

Results and conclusions: Eighty-two urine samples screened as positive for rods from Sysmex UF-1000i were positive on classic method (≥ 1 x 105 bacteria/mL). Regarding quantification, significant differences ($p = 0.013$) between Sysmex UF-1000i and routine were found however not exceeding ± 1 log, with no clinical meaning. MALDI-TOF was able to identify directly from urine (at least in one spot) 95.3% of the isolates, being the majority (70.4%) *E. coli*, *Kl. pneumoniae* (8.2%), *Proteus mirabilis* (3.3%), Enterobacter spp. (6.6%), *Pseudomonas aeruginosa*, *Providencia* and *Aeromonas* (1 isolate for each). The 4 cases (6.6%) without identification directly, did not had pellet after extraction protocol (1 Salmonella, 3 E. coli). The PA between the identifications obtained directly from urines and from colonies was 100%. Therefore, 57 samples analyzed on Sysmex weren't considered for identification on MALDI-TOF, because they were negative (14), showed cocci/mixed population (22) or presented values > 1 x 105 bacteria/mL but without clarifying if they were rods or cocci/mixed (21).

In conclusion, the Sysmex UF-1000i revealed to be a useful tool for screening and quantification positive urines. Additionally, MALDI-TOF directly from urine showed to be accurate and fast (few minutes versus 24h), representing a step forward on UTIs diagnosis.

Keywords: Urinary tract infections; Gram-negative bacilli; Flow cytometry; Mass spectrometry