

Article



# **Comparative Evaluation of Bruker Biotyper and ASTA MicroIDSys for Species Identification in a Clinical Microbiology Laboratory**

Yousun Chung<sup>1</sup>, Minje Han<sup>1</sup> and Jae-Seok Kim<sup>1,2,\*</sup>

- <sup>1</sup> Department of Laboratory Medicine, Kangdong Sacred Heart Hospital, Seoul 05355, Korea; yousun623@kdh.or.kr (Y.C.); mjhan@kdh.or.kr (M.H.)
- <sup>2</sup> Department of Laboratory Medicine, Hallym University College of Medicine, Chuncheon 24252, Korea
- \* Correspondence: jaeseok@hallym.ac.kr; Tel.: +82-2-2224-2327

Abstract: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been widely used for microbial identification, because of its speed and accuracy, since its introduction to clinical microbiology laboratories. In this study, we evaluated the performance of ASTA MicroIDSys, a newly developed MALDI-TOF, and compared it with the widely used Bruker Biotyper. Microbial identification with the Bruker Biotyper system was performed by using a direct smear method and the Bruker Biotyper database (reference library version 6.0.0.0). The isolates were also tested in parallel, using the ASTA MicroIDSys system with a direct smear method and the MicroIDSys database, CoreDB v1.26. A total of 914 clinical isolates were recovered from the clinical specimens. Identical results with confidence scores ( $\geq 2.0$ , for the Bruker Biotyper) and acceptable scores (≥140 for the ASTA MicroIDSys) were obtained for 840 (91.9%) isolates. The minor errors were defined as misidentification at the species level, and the rate was 1.1% (9/792) for Bruker Biotyper and 1.6% (13/792) for ASTA MicroIDSys. Major errors were defined as misidentification at the genus level, and the rate was 0.3% (2/792) for both Bruker Biotyper and ASTA MicroIDSys. ASTA MicroIDSys showed reliable performance for microbial identification, which was comparable to that of the Bruker Biotyper. Therefore, ASTA MicroIDSys can be applied for the identification of microorganisms in clinical microbiology laboratories.

**Keywords:** matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS); microorganism; identification

# 1. Introduction

The introduction of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS) has greatly improved the turnaround time for the routine identification of microorganisms, along with rapid and accurate identification [1–3]. MALDI–TOF MS provided consistent and accurate results when compared with those of biochemical identification methods, but no standard method for dealing with unexpected species identification has been presented. The discrepancies in the outcomes for rare bacterial species for different MALDI–TOF MS manufacturers should be examined.

There are two commercially available MALDI–TOF MS systems: the Microflex Biotyper (Bruker Daltonics, Bremen, Germany) and VITEK MS (bioMérieux, Marcy l'Etoile, France), which are implemented in clinical microbiology laboratories worldwide [4–7]. Recently, a new MALDI–TOF MS system, ASTA MicroIDSys (ASTA Inc., Suwon, Korea), was developed for the identification of clinically important microorganisms.

In the present study, we compared the routine performance of the ASTA MicroIDSys with that of the Microflex Biotyper for identification of all microbial isolates, including bacteria and yeasts, in a clinical microbiology laboratory, during the study period.



**Citation:** Chung, Y.; Han, M.; Kim, J.-S. Comparative Evaluation of Bruker Biotyper and ASTA MicroIDSys for Species Identification in a Clinical Microbiology Laboratory. *Diagnostics* **2021**, *11*, 1683. https:// doi.org/10.3390/diagnostics11091683

Academic Editor: Georgina Tzanakaki

Received: 12 August 2021 Accepted: 14 September 2021 Published: 15 September 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/).

### 2. Materials and Methods

All clinical strains, except for filamentous fungi and mycobacteria, isolated in a clinical microbiology laboratory at a 750-bed general hospital in Korea, from October to December 2018, were included in this study. The study was approved by the institutional review board of Kangdong Sacred Heart Hospital (IRB file No. Kangdong NON2018-001, 18 September 2018). The clinical specimens were inoculated in appropriate media, such as 5% sheep blood agar, MacConkey agar, or chocolate agar for bacteria; Buccella blood agar for anaerobic bacteria; and Sabouraud dextrose agar for yeast. The specimens were then incubated for 24–48 h, at 35 °C, in appropriate conditions. A total of 914 clinical isolates were recovered from clinical specimens of blood, body fluids, wounds, and pus.

Microbial identification with Bruker Biotyper system was performed by using a direct smear method, following the manufacturer's instructions. Briefly, a singly colony of the isolate was smeared and dried on a plate. Subsequently, 70% formic acid and cyano-4-hydroxycinnamic acid matrix solution were added, and the target plate was analyzed by using Bruker Biotyper database (reference library version 6.0.0.0, Bruker Daltonics, Bremen, Germany). The confidence scores values over 2.0 were considered acceptable according to the manufacturer's recommendations. The isolates were also tested in parallel, using ASTA MicroIDSys system with a direct smear method. Moreover, the target plate was analyzed by using MicroIDSys database (CoreDB v1.26, ASTA Inc., Suwon, Korea). Identification scores over 140 were considered acceptable according to the manufacturer's recommendations. If the test provided a score under the target cutoff (<140 for MicroIDSys and <2.0 for Bruker Biotyper) or invalid results, we immediately repeated the test with other colonies from the same agar plate.

When the results by two systems showed discrepancies at the species level or one of the results was under the cutoff score or invalid, 16S rRNA gene sequencing was performed for bacterial identification by Macrogen (Seoul, Korea). The PCR primers for 16S rRNA were 5'-GGATTAGATACCCTGGTA-3' and 5'-CCGTCAATTCMTTTRAGTTT-3', and the sequencing primers were 5'-AGAGTTTGATCMTGGCTCAG-3' and 5'-TACGGYTACCTT GTTACGACTT-3'. The 16S rRNA sequences obtained were compared with GenBank data, using the BLAST alignment software (blast.ncbi.nlm.nih.gov, accessed on 12 July 2021) and a threshold of  $\geq$ 99% homology was used for identification to the species level.

Chi-square test or Fisher's exact test was used for analyzing differences in the identification rate. SPSS Statistics 24 (IBM SPSS Inc., Chicago, IL, USA) was used for statistical analyses, and a 2-tailed *p*-value of 0.05 was considered statistically significant.

# 3. Results

The isolates were identified as belonging to Gram-negative bacilli (N = 417, 45.6%), Gram-positive cocci (N = 329, 36.0%), and other bacteria (N = 60, 6.6%), and fungi (N = 108, 11.8%). The most frequently isolated bacteria were *Escherichia coli* (N = 136, 14.9%), followed by *Staphylococcus aureus* (N = 99, 10.8%), *Klebsiella pneumoniae* (N = 79, 8.6%), *Enterococcus faecium* (N = 63, 6.9%), *Acinetobacter baumannii* (N = 48, 5.3%), *Pseudomonas aeruginosa* (N = 46, 5.0%), *Candida tropicalis* (N = 42, 4.6%), *Candida albicans* (N = 36, 3.9%), *E. faecalis* (N = 33, 3.6%), *S. epidermidis* (N = 25, 2.7%), *Corynebacterium striatum* (N = 25, 2.7%), *Candida glabrata* (N = 19, 2.1%), *S. haemolyticus* (N = 18, 2.0%), *Enterobacter aerogenes* (N = 15, 1.6%), and *Streptococcus anginosus* (N = 14, 1.5%).

From the 914 isolates analyzed, identical results with confidence scores ( $\geq$ 2.0 for the Bruker Biotyper) and acceptable scores ( $\geq$ 140 for the ASTA MicroIDSys) were obtained for 840 (91.9%) isolates. After applying lower confidence scores ( $\geq$ 1.7) for the Bruker Biotyper, 24 (94.5%) additional isolates showed identical results for the two systems (Table 1).

Species         ASTA $\geq 140$ ASTA $< 140$		Bruker $\geq$ 2.0		<b>Bruker 1.7</b> ≤, <2.0		
Gram-negative bacilli         134         1 $1^{*1}$ 136           Klebsila preumoniae         79         79         79           Actinetobacter baumanutii         48         48           Pseudomons aeruginosa         45         1         46           Protous miratiliis         18         18         18           Enterobacter acogenes         15         15         15           Enterobacter acogenes         8         8         8           Stantorphomonas malcophilia         7         7         14           Actineobacter bacypit         2         2         1           Actineobacter bacypit         2         2         2           Alcatigenes facatils         2         2         2           Alcatigenes facatils         2         2         2           Actineobacter stylosoxidans         2         2         3           Actineoba	Species	$ASTA \ge 140$	ASTA < 140	$ASTA \ge 140$	ASTA < 140	- Total
Escherichia coli         134         1 $1^{*1}$ 136           Klebsidla preemoniae         79	Gram-negative bacilli					
Klesicfla presumoniae         79         1         70           Actinetobacer bummitii         48         48           Pseudomona aeruginosa         45         1           Hornotecter aerogenes         15         15           Enterobacter cloacae         9         9           Serratin marcescenes         8         8           Stenotrophomous multophilia         7         7           Haemophilus influenzae         5         2           Chrobacter founditis         1         4           Actinetobacter buyli         3*2         3           Providencia retrigeri         2         2           Actinetobacter noncominitis         2         2           Achromobacter xylosoxidans         2         2           Achromobacter xylosoxidans         2         2           Achromobacter pittii         1         1*3         2           Providencia stutzeri         1         1         1           Actinetobacter pittii         1         1         1           Samomolas stutzeri         1         1         1           Achromobacter yioni         1         1         1           Achromobacter pittii         1         1 </td <td>Escherichia coli</td> <td>134</td> <td>1</td> <td></td> <td>1 * 1</td> <td>136</td>	Escherichia coli	134	1		1 * 1	136
Anterbaker bummuti       48       48         Pseudomonas arenginosa       45       1       46         Protes minbilis       18       18       18         Enterobacter clonce       9       9       9         Serratia marcescens       8       8       5         Stenotrophonons multophila       7       14       4         Acinetobacter haplyi       3*2       3       2         Cirtobacter freundi       4       4       4         Acinetobacter haplyi       3*2       2       2         Arcinetobacter malonaticus       2       2       2         Actinetobacter malonaticus       2       2       2         Actinetobacter malonaticus       2       2       2         Actinetobacter noscomialis       2       2       2         Actinetobacter noscomialis       2       2       2         Actinetobacter subtriti       1       1       1       1         Salmonella spp.       1       1       1       1         Providencia stuarti       1       1       1       1         Providencia stuarti       1       1       1       1         Actinetobacter piti	Klebsiella pneumoniae	79	-		1	79
Pseudomons aeruginosa         45         1         46           Proteus minbilis         18         18           Entrobacter aerogenes         15         15           Entrobacter aerogenes         16         9           Serratia marcescene         8         8           Strontorphomonas mallophilla         7         7           Haemophilus influenzae         5         2           Chrobacter foundii         4         4           Acinetobacter buly in         3 * 2         3           Providencia rettgeri         2         2           Alcatigenes foccultis         2         2           Actronobacter zylosoxidans         2         2           Actronobacter zylosoxidans         2         2           Actronobacter zylosoxidans         2         1           Actronobacter zylosoxidans         1         1           Campobacter zylosoxidans         1         1           Actronobacter zylosoxidans	Acinetobacter baumannii	48				48
Proteus minubils1818Enterobacter acrogenes1515Enterobacter cloaca9Serratia marcsecens8Stenotrephononus maltophila7Haemophilus influenzae5Cirrobacter freundi4Acinetobacter buylyi $3^{*2}$ Acinetobacter buylyi $2^{*2}$ Acinetobacter andlogeni2Morganella morganii2Cirrobacter malonaticus2Achietobacter noscomialis2Achietobacter noscomialis2Achietobacter noscomialis2Achietobacter subscriptini1Salmonella spp.1Achietobacter subscriptini1Salmonella spp.1Achietobacter subscriptini1Salmonella spp.1Acromonas caviae1I1Providencia stuarti1I1Providencia stuarti1I1Brezundinonas survise1I1Brezundinonas caviae1I1No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)I (0.2%)401 (100%)Gram-positive cocci25Staphylococcus agalactiae2I (100%)4Staphylococcus agalactiae2I (100%)3Staphylococcus agalactiae2I (100%)3Staphylococcus agalactiae2I (100%)2Staphylococcus agalactiae </td <td>Pseudomonas aeruginosa</td> <td>45</td> <td></td> <td>1</td> <td></td> <td>46</td>	Pseudomonas aeruginosa	45		1		46
Enterobacter elocate1515Enterobacter clocate99Serratia marcescens8Steutorphomous maliophilia7Haemophilis influenze5Citrobacter freundii4Acinetobacter bulyi $3^{*2}$ Providencia retigeri2Arangenes faccalis2Morganella morganii222Actirobacter subsocialans2Actirobacter subsocialans2Actirobacter subsocialans2Actirobacter subsocialans2Actirobacter subsocialans2Actirobacter subsocialans1Actirobacter subsocialans1Actirobacter subsocialans1Actirobacter subsocialans1In Salmonella spr.1Actirobacter printi1Netsseria gonorrhoeae111Providencia stuartii111Providencia stuartii111Remophilus parainfiluenza111Remophilus parainfiluenza1111111111111111221111221111221112133333	Proteus mirabilis	18				18
$ \begin{array}{ccccc} Entrobacter close & 9 & 9 \\ Serratia marcescens & 8 & 8 \\ Stenotrophomous multophilia & 7 \\ Haemophilus influenzae & 5 \\ Cirrobacter freinuli & 4 & 3 + 2 & 3 \\ Acinetobacter baylyi & 3 + 2 & 2 \\ Alcaligenes faccalis & 2 & 2 \\ Alcaligenes faccalis & 2 & 2 \\ Alcaligenes faccalis & 2 & 2 \\ Cirrobacter analonaticus & 2 & 2 \\ Achinetobacter noscontalis & 1 & 1 \\ Salmonella spp. & 1 & 1 \\ Achinetobacter noscontalis & 2 \\ Achinetobacter noscontalis & 3 \\ Stephylococcus anceus & 99 \\ Entencoccus agalactiae & 1 \\ Achinetobacter noscontalis & 2 \\ Stephylococcus antimis & 1 \\ Achinetobacter & 2 \\ Achinetoba$	Enterobacter aerogenes	15				15
Serratia marcescens88Stenotrophomous maltophilia77Haemophilus influenzae55Citrobacter freundii44Acinetobacter baylyi $3^{+2}$ 3Providencia religeri22Morganella morganii22Citrobacter amalonaticus22Achromobacter xylosoxidans22Achromobacter rylosoxidans22Achromobacter printi11*32Providencia sturiti11*32Providencia sturiti111Salmonella spp.111Acromobacter printi111Providencia sturiti111Providencia sturiti111Providencia sturiti111Providencia sturiti111Providencia sturiti111Providencia sturiti111Providencia sturiti111Acromonas croiti111In Aeromonas croiti111Campylobacter jejuni111Hencophilus parainfluenzae111Staphylococcus facium632525Staphylococcus facium333Staphylococcus andy fictus111Staphylococcus andy fictus112Staphylococcus andy fictus22<	Enterobacter cloacae	9				9
$\begin{array}{cccccc} Steed reprint Stee$	Serratia marcescens	8				8
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Stenotrophomonas maltophilia	7				7
$ \begin{array}{c} Ctrobucter baylyi & 3 * 2 & 3 \\ Acinetobacter baylyi & 3 * 2 & 3 \\ Providencia rettgeri & 2 & 2 \\ Alcaligenes facalis & 2 & 2 \\ Morganella morganis & 2 & 2 \\ Ctrobucter analomaticus & 2 & 2 \\ Achronobacter analomaticus & 2 & 2 \\ Achronobacter analomaticus & 2 & 2 \\ Achronobacter moscomialis & 2 & 2 \\ Achronobacter pittii & 1 & 1 * ^3 & 2 \\ Providencia stuartii & 1 & 1 \\ Salmonella spp. & 1 & 1 \\ Aeromonas veronii & 1 & 1 \\ Pesculamonus stutzeri & 1 & 1 \\ Neisseria gonorrhoeae & 1 & 1 \\ Aeromonas croiae & 1 & 1 \\ Brevundinonus stutzeri & 1 & 1 \\ Haemophilus parainfluenzae & 1 & 1 \\ Brevundinonus vesculatris & 1 & 1 \\ No. of subtotal (%) & 393 (98.0%) & 1 (0.2%) & 6 (1.5%) & 1 (0.2%) & 401 (100%) \\ Gram-positive cocci & 99 \\ Enterooccus faccium & 63 & 63 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus aureus & 99 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus sureus & 99 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus sureus & 99 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus sureus & 99 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus sureus & 99 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus sureus & 99 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus sureus & 99 \\ Enterooccus faccium & 63 & 33 \\ Staphylococcus sureus & 99 \\ Staphylococcus sureus & 99 \\ Staphylococcus sureus & 90 & 10 & 4 \\ Staphylococcus sureus & 91 & 9 \\ Staphylococcus sureus & 2 & 7 \\ Furterooccus faccium & 5 & 2 & 7 \\ Staphylococcus sureus & 33 \\ Staphylococcus sureus & 2 & 2 \\ Streptococcus casselflatus & 4 & 4 \\ Staphylococcus sureus & 2 & 2 \\ Streptococcus casselflatus & 2 & 2 \\ Streptococcus sureus & 2 & 2 \\ Streptococcus sureus & 2 & 2 \\ Streptococcus sureus & 1 & 1 \\ Streptococcus sur$	Haemophilus influenzae	5				5
Acinetobacter baylyi $3^{*2}$ 3Providencia rettyeri22Alcailgenes facculis22Morganiella morganii22Citrobacter malonaticus22Achromobacter xylosoxidams22Achromobacter sylosoxidams22Achromobacter sylosoxidams22Achromobacter sylosoxidams22Achromobacter sylosoxidams11Providencia stuartii11I11Salmonella spp.11Aeromonas veronii11Aeromonas veronii11Hearonofilus gonorrhoene11Haenophilus parainfluenzae11I11Brezundinonas vesicularis1*41Brezundinonas vesicularis3333Staphylococcus aureus9999Enterococcus facculis3333Staphylococcus aureus9925Staphylococcus agrinosus19Staphylococcus agrinosus19Staphylococcus agrinosus77Staphylococcus agrinosus27Enterococcus retikeris33Straphylococcus sugrinosus27Enterococcus agrinosus27Enterococcus agrinosus22Staphylococcus agrinosus22Enterococcus agrinosus22Staphylococcus agrinosus22 <tr< td=""><td>Citrobacter freundii</td><td>4</td><td></td><td></td><td></td><td>4</td></tr<>	Citrobacter freundii	4				4
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Acinetobacter baylyi			3 * <sup>2</sup>		3
Alcaligenes faccalis2Morganella morganii2Citrobacter analonaticus2Achronobacter xylosoxidans2Acinetobacter nosocomialis2Acinetobacter nosocomialis2Acinetobacter nosocomialis2Acinetobacter pittii111*32Providencia stuarii11Actinetobacter pittii111Actinetobacter pittii111Aeromonas sturzeri111Preudomonas sturzeri111Aeromonas caviae111Campulobacter jejuni111Brezundimonas obscicularis1*411No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)401 (100%)Gram-positive cocci25Staphylococcus squerus99Enterococcus faecalis33Staphylococcus anginosus10414Staphylococcus anginosus10414Staphylococcus lominitis819Staphylococcus lominitis27Staphylococcus lominitis81921212121212121211212 <td>Providencia rettgeri</td> <td>2</td> <td></td> <td></td> <td></td> <td>2</td>	Providencia rettgeri	2				2
$\begin{array}{c cccc} Marganiala morganii & 2 & 2 \\ Citrobacter malonaticus & 2 & 2 \\ Achromobacter xylosoxidans & 2 & 2 \\ Achromobacter ylosoxidans & 2 & 2 \\ Achromobacter yliti & 1 & 1 * ^3 & 2 \\ Providencia stuartii & 1 & 1 * ^3 & 2 \\ Providencia stuartii & 1 & 1 * ^3 & 2 \\ Providencia stuartii & 1 & 1 & 1 \\ Salmonella spp. & 1 & 1 \\ Aeromonas veronii & 1 & 1 \\ Pseudomonas stutzeri & 1 & 1 \\ Neisseria gonorrhocae & 1 & 1 \\ Acromonas carviae & 1 & 1 \\ Campulobacter jejuni & 1 & 1 \\ Haemophilus parainfluenzae & 1 & 1 \\ Brevundimonas vesicularis & 1 * ^4 & 1 \\ No. of subtotal (%) & 393 (98.0%) & 1 (0.2%) & 6 (1.5\%) & 1 (0.2\%) & 401 (100\%) \\ Gram-positive cocci & 1 & 63 & 63 \\ Enterococcus fueciun & 63 & 63 \\ Enterococcus sureus & 99 & 99 \\ Enterococcus sureus & 99 & 99 \\ Enterococcus sureus & 14 & 4 & 18 \\ Streptococcus anemolyticus & 14 & 4 & 14 \\ Streptococcus andentia & 1 & 9 \\ Staphylococcus capitis & 7 & 7 \\ Staphylococcus capitis & 7 & 7 \\ Staphylococcus capitis & 7 & 3 \\ Staphylococcus capitis & 7 & 3 \\ Streptococcus anginosus & 1 & 1 & 2 \\ Streptococcus anginosus & 1 & 1 & 2 \\ Streptococcus anginosus & 1 & 1 & 2 \\ Streptococcus sureus & 2 & 7 \\ Enterococcus sureus & 4 & 14 \\ Streptococcus sureus & 9 & 2 & 2 \\ Staphylococcus capitis & 7 & 3 \\ Staphylococcus capitis & 7 & 3 \\ Staphylococcus capitis & 7 & 3 \\ Streptococcus sureus & 4 & 3 \\ Streptococcus sureus & 4 & 3 \\ Streptococcus sureus & 2 & 2 \\ Streptococcus sureus & 2 & 2 \\ Streptococcus sureus & 1 & 1 & 2 \\ Streptococcus sureus & 1 & 1 & 2 \\ Streptococcus sureus & 1 & 1 & 2 \\ Streptococcus sureus & 1 & 1 & 1 \\ Streptococcus sureus & 1 & 1 & 1 \\ Streptococcus sureus & 1 & 1 \\ Streptococcus sure$	Alcaligenes faecalis	2				2
$\begin{array}{ccccc} Citrobacter analomaticus & 2 & 2 \\ Achronobacter aylosoxidans & 2 & 2 \\ Achrotobacter nosoconialis & 2 & 2 \\ Achrotobacter pitti & 1 & 1*^3 & 2 \\ Providencia stuartii & 1 & 1*^3 & 2 \\ Providencia stuartii & 1 & 1 \\ Salmonella spp. & 1 & 1 \\ Aeromonas veronii & 1 & 1 \\ Pseudomonas stuzeri & 1 & 1 \\ Neisseria gonorrhoeae & 1 & 1 \\ Aeromonas caviae & 1 & 1 \\ Campylobacter jejuni & 1 & 1 \\ Haemophilus parainfluenzae & 1 & 1 \\ Breundinonas versicularis & 1*^4 & 1 \\ No. of subtotal (%) & 393 (98.0%) & 1 (0.2%) & 6 (1.5%) & 1 (0.2%) & 401 (100%) \\ Gram-positive cocci & 99 & 99 \\ Enterococcus facium & 63 & 63 \\ Enterococcus equidermidis & 25 & 25 \\ Staphylococcus aneus & 99 & 99 \\ Enterococcus agalactiae & 10 & 10 \\ Staphylococcus aneus & 14 & 4 \\ Streptococcus agalactiae & 10 & 10 \\ Staphylococcus aneus & 1 & 9 \\ Staphylococcus aguinensis & 7 & 7 \\ Staphylococcus aguinensis & 7 & 7 \\ Staphylococcus aguinensis & 7 & 7 \\ Staphylococcus aguinensis & 7 & 3 \\ Streptococcus aguinensis & 7 & 3 \\ Streptococcus aguinensis & 7 & 3 \\ Streptococcus aguinensis & 7 & 2 \\ Staphylococcus aguinensis & 7 & 2 \\ Staphylococcus aguinensis & 7 & 2 \\ Staphylococcus aguinensis & 7 & 2 \\ Streptococcus aguinensis & 2 & 2 \\ Streptococcus aguinensis & 2 & 2 \\ Streptococcus aguinensis & 1 & 1 & 2 \\ Streptococcus aguinensis & 1 & 1 & 2 \\ Streptococcus aguinensis & 1 & 1 & 2 \\ Streptococcus aguinensis & 1 & 1 \\ Micrococcus aguinensis & 1 & 1 \\ Streptococcus aguinensis & 1 & 1 \\ Streptococcus aguinarius & 1 & 1 \\ Streptococcus aguinins & 1 & 1 \\ Streptococcu$	Morganella morganii	2				2
Actromobacter nosocomialis22Acinetobacter nosocomialis22Acinetobacter pittii1 $1^{*3}$ 2Providencia stuartii111Salmonella spp.111Aeromonas veronii111Pseudomonas stutzeri111Neisesiri agonorhoeae111Idemonphilus parainfluenzae111Idemonphilus parainfluenzae111Idemonphilus parainfluenzae111No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)Gram-positive cocci1133Staphylococcus aureus99996Enterococcus aureus99663Staphylococcus aureus993333Staphylococcus aureus9910414Streptococcus aginosus1041414Streptococcus agiliciae101093Staphylococcus agiliciae7733Streptococcus agiliciae227Enterococcus agiliciaus422Streptococcus agiliciaus222Streptococcus agiliciaus112Streptococcus agiliciaus112Streptococcus militis221Streptococcus militis112Streptococcus militis<	Citrobacter amalonaticus	2				2
Acinetobacter pittii       2         Acinetobacter pittii       1         Salmonella spp.       1         Salmonella spp.       1         Aeromonas veronii       1         Providencia stuartii       1         Aeromonas veronii       1         Preudomonas veronii       1         Preudomonas veronii       1         Preudomonas veronii       1         Neiseria gonorthoeae       1         Image: Construction of the system of t	Achromobacter xylosoxidans	2				2
Acinetobacter pittii1 $1^{*3}$ 2Providencia stuartii11Salmonella spp.1Aeromonas sevennii1Insesseria gonorrheae1Neisseria gonorrheae1Campulobacter jejuni1Haemophilus parainfluenzae1Itamophilus parainfluenzae9Staphylococcus aureus99Enterococcus faecalis33Staphylococcus haemolyticus1Itamophilus Staphylococcus haemolyticus1Itamophilus Staphylococcus anglactiae10Staphylococcus anglactiae10Staphylococcus anglactiae2Itamophilus Caspate2Streptococcus anglinosus2Streptococcus mitis2Streptococcus mitis1Itamophilus Simulans1Itamophilus Caspate2Streptococcus mitis2Streptococcus mitis1Streptococcus mitis </td <td>Acinetobacter nosocomialis</td> <td>2</td> <td></td> <td></td> <td></td> <td>2</td>	Acinetobacter nosocomialis	2				2
Providencia stuartii       1       1         Salmonella spp.       1       1         Aeromonas veronii       1       1         Pseudomonas stutzeri       1       1         Neisseria gonorrhoeae       1       1         Aeromonas caviae       1       1         Campylobacter jejuni       1       1         Haemophilus parainfluenzae       1       1         Breoundimonas vesicularis       1 (0.2%)       6 (1.5%)       1 (0.2%)         Gram-positive cocci       1       1       1         Staphylococcus aureus       99       99       99       1 (0.2%)       401 (100%)         Gram-positive cocci       333       33       33       33       33       33         Staphylococcus faeciain       63       25       5       5       5       5       5       5       5       5       10       10       14       14       14       14       14       14       14       14       14       14       14       14       14       15       10       10       10       10       10       10       10       10       10       11       1       11       11       11       <	Acinetobacter pittii	1		1 * <sup>3</sup>		2
Salmonella spp.11Aeromonas veronii11Pseudomonas stutzeri11Neisseria gonorrhoeae11Aromonas caviae11Campylobacter jejuni11Haemophilus parainfluenzae11Brevundimonas vesicularis $1^{*4}$ 1No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)Gram-positive cocci9999Enterococcus aureus996Staphylococcus aureus996Enterococcus faecalis3333Staphylococcus aginosus10414Streptococcus agalactiae10100Staphylococcus logitis77Staphylococcus capitis77Staphylococcus capitis77Staphylococcus capitis73Streptococcus capitis72Enterococcus capitis72Strephococcus agifurosus44Strephococcus capitis72Strephococcus capitis22Enterococcus capitis22Streptococcus agifurosus22Streptococcus alizarius11221Streptococcus alizarius11Streptococcus alizarius11Streptococcus alizarius11Streptococcus alizarius11Micrococcus alizarius11Micrococcus ali	Providencia stuartii	1				1
Aeromonas veronii11Pseudomonas stutzeri11Neisseria gonorrhocae11Aeromonas caviae11Campylobacter jejuni11Haemophilus parainfluenzae11Brevundimonas vesicularis1*41No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)Gram-positive cocci99Enterococcus faecium6363Enterococcus faecialis3333Staphylococcus equipermidis2525Staphylococcus agalactiae1010Staphylococcus longuinesis77Staphylococcus longuinesis77Staphylococcus longuinesis77Staphylococcus longuinesis77Staphylococcus capitis77Staphylococcus longinesis44Steptococcus agalactiae19Staphylococcus soprate52Enterococus capitis733Streptococus capitas22Enterococcus agaliau22Enterococcus agaliau22Enterococcus aditis11227Staphylococcus singuinesis11333Streptococcus andfarosu22Enterococcus aditis11Streptococcus aditis11Streptococcus alivanis11Micrococcus silvanis1	Salmonella spp.	1				1
Pseudomonas stutzeri11Neisseria gonorrhoeae11Aeromonas caviae11Campylobacter jejuni11Haemophilus parainfluenzae11Breoundimonas resicularis $1^{*4}$ 1No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)Gram-positive cocci199Enterococcus faccium6363Enterococcus faccium6363Enterococcus faccium2525Staphylococcus anginosus104Streptococcus agalactiae10Staphylococcus agalactiae10Staphylococcus caprae7Staphylococcus caprae7Staphylococcus caprae2Fenterococcus casseliflavus4Streptococcus avium2Streptococcus avium2Streptococcus altis112Streptococcus avium2Streptococcus altis2Streptococcus altis2Streptococcus altis121Streptococcus altis132Streptococcus altis112Streptococcus altis112Streptococcus altis111Streptococcus altis111Streptococcus altis111Streptococcus altis111Streptococcus altis1	Aeromonas veronii	1				1
Neisseria gonorthoeae11Aeromous caviae11Campylobacter jejuni11Haemophilus parainfluenzae11Brevundimonas vesicularis $1^{*4}$ 1No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)401 (100%)Gram-positive cocci $1^{*4}$ 16363Enterococcus faccium636363Enterococcus faccialis333333Staphylococcus epidermidis2525Staphylococcus haemolyticus14418Streptococcus faccialis10414Streptococcus agalactiae1010Staphylococcus lugdunensis77Staphylococcus capitis77Staphylococcus capitis77Staphylococcus capitis73Streptococcus acaitis22Enterococcus asseliflavus42Streptococcus alturius112Streptococcus alturius2Streptococcus alturius112Streptococcus alturius1112Streptococcus salivarius11Streptococcus salivarius11Streptococcus salivarius11Streptococcus salivarius11Streptococcus salivarius11Streptococcus salivarius11Streptococcus salivarius11Streptoco	Pseudomonas stutzeri	1				1
Aeromonas caviae11Campylobacter jejuni11Haemophillus parainfluenzae1Brevundimonas vesicularis $1*4$ 1No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)401 (100%)Gram-positive cocci $1*4$ 11Staphylococcus aureus9999Enterococcus faccium6363Enterococcus faccilis3333Staphylococcus anginosus104Streptococcus anginosus104Streptococcus anginosus104Streptococcus anginosus7Staphylococcus lugdunensis7Staphylococcus caprae52Streptococcus caprae52Enterococcus avium2Streptococcus avium2Streptococcus avium2Streptococcus avium2Streptococcus sollatus2Streptococcus sollatus2Streptococcus sollatus2Streptococcus sollatus2Streptococcus sollatus2Streptococcus sollatus112Streptococcus sollatus112Streptococcus salivarius112Streptococcus simulans1Micrococcus luteus1Streptococcus simulans1Streptococcus simulans1Streptococcus simulans1Streptococcus luteus1Streptococcus simulans1 <td>Neisseria gonorrhoeae</td> <td>1</td> <td></td> <td></td> <td></td> <td>1</td>	Neisseria gonorrhoeae	1				1
Campylobacter jejuni       1       1         Haemophilus parainfluenzae       1       1         Brevundimonas vesicularis       1*4       1         No. of subtotal (%)       393 (98.0%)       1 (0.2%)       6 (1.5%)       1 (0.2%)       401 (100%)         Gram-positive cocci       99       99       99       99         Enterococcus faecium       63       63       63         Enterococcus faecius       33       33       33         Staphylococcus epidermidis       25       25       5         Staphylococcus agalactiae       10       4       14         Streptococcus agalactiae       10       10       34       14         Streptococcus agalactiae       10       10       35       34       34         Streptococcus agalactiae       10       10       34       34       34       34       35       35       35       35       36       36       36       36       36       36       36       36       36       36       36       36       36       36       36       36       36       36       37       37       37       35       36       36       36       36       36 <t< td=""><td>Aeromonas caviae</td><td>1</td><td></td><td></td><td></td><td>1</td></t<>	Aeromonas caviae	1				1
Haemophilus parainfluenzae11Brevundimonas vesicularis $1^{*4}$ 1No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)401 (100%)Gram-positive cocci $5taphylococcus aureus$ 9999Enterococcus faecalis3333Staphylococcus aureus9963Enterococcus faecalis3333Staphylococcus haemolyticus144Streptococcus agalactiae1040Staphylococcus lugdunensis77Staphylococcus capate52Staphylococcus caprae52Enterococcus genes27Enterococcus genes27Enterococcus raginosus19Staphylococcus caprae52Staphylococcus caprae52Enterococcus genes22Enterococcus raginosus11Streptococcus aritis22Streptococcus aritis22Streptococcus pogenes22Streptococcus silvarius11Streptococcus silvarius11Micrococcus sinuans11Micrococcus silvarius11Streptococcus sinuans11Streptococcus intermedius11Streptococcus intermedius11Streptococcus intermedius11Streptococcus intermedius11Streptococcus intermedius11 </td <td>Campylobacter jejuni</td> <td>1</td> <td></td> <td></td> <td></td> <td>1</td>	Campylobacter jejuni	1				1
Brevundimonas vesicularis $1*4$ 1No. of subtotal (%)393 (98.0%)1 (0.2%)6 (1.5%)1 (0.2%)401 (100%)Gram-positive cocci $5taphylococcus aureus$ 9999Enterococcus faecium6363Enterococcus faecius3333Staphylococcus epidermidis2525Staphylococcus aginosus10414Streptococcus aginosus10414Streptococcus aginosus10414Streptococcus aginosus1077Staphylococcus capitis777Staphylococcus capitis77Staphylococcus pogene527Enterococcus ayeginas4433Streptococcus pogenes272Enterococcus avitis222Streptococcus pugenes222Streptococcus avitis222Streptococcus avitis222Streptococcus avitis222Streptococcus suitis222Streptococcus avitis112Streptococcus sinutins111Micrococcus sinutans111Streptococcus sinutans111Streptococcus sinutans111Streptococcus pogeneting111Streptococcus sinutans111Streptococcus sinutans1 <td>Haemophilus parainfluenzae</td> <td>1</td> <td></td> <td></td> <td></td> <td>1</td>	Haemophilus parainfluenzae	1				1
No. of subtotal (%)       393 (98.0%)       1 (0.2%)       6 (1.5%)       1 (0.2%)       401 (100%)         Gram-positive cocci	Brevundimonas vesicularis			$1 * {}^{4}$		1
Gram-positive cocciStaphylococcus aureus9999Enterococcus faccium6363Enterococcus faccilis3333Staphylococcus epidermidis2525Staphylococcus anginosus144Streptococcus anginosus104Streptococcus anginosus1010Staphylococcus hominis81Staphylococcus capitis77Staphylococcus capitis77Staphylococcus capitis77Staphylococcus anginosus44Staphylococcus capitis77Staphylococcus capitis72Enterococcus capitis73Streptococcus pogenes22Enterococcus rollatus22Streptococcus anitis22Streptococcus sultus12Streptococcus sultus12Streptococcus sultus11Streptococcus sulturius11Micrococcus sulturius11Streptococcus sulturius11Streptococcus sulturius11Streptococcus intermedius11Streptococcus intermedius11Streptococcus intermedius11Streptococcus sulturius11Streptococcus intermedius11Streptococcus intermedius11Streptococcus intermedius11Streptococcus intermedius <td< td=""><td>No. of subtotal (%)</td><td>393 (98.0%)</td><td>1 (0.2%)</td><td>6 (1.5%)</td><td>1 (0.2%)</td><td>401 (100%)</td></td<>	No. of subtotal (%)	393 (98.0%)	1 (0.2%)	6 (1.5%)	1 (0.2%)	401 (100%)
Staphylococcus aureus9999Enterococcus faccium6363Enterococcus faccilis33Staphylococcus epidermidis25Staphylococcus epidermidis25Staphylococcus anginosus10414Streptococcus anginosus10104Staphylococcus lugdunensis7Staphylococcus capitis77Staphylococcus capitis75Staphylococcus capitis775Staphylococcus capitis775Staphylococcus pettenkoferi333Streptococcus anitis222Enterococcus capital222Enterococcus capital222Enterococcus capital233335222Enterococcus capital233333333522222223232323233334452225223333333445252 <td>Gram-positive cocci</td> <td></td> <td></td> <td></td> <td></td> <td></td>	Gram-positive cocci					
Enterococcus faecuim6363Enterococcus faecalis33Staphylococcus epidermidis25Staphylococcus naemolyticus144Streptococcus anginosus10414Streptococcus anginosus1010Staphylococcus hominis819Staphylococcus capalactiae1010Staphylococcus lugdunensis77Staphylococcus capitis777Staphylococcus caprae527Enterococcus pogenes222Enterococcus rulim222Streptococcus suitis222Enterococcus rulim222Streptococcus sonstellatus222Streptococcus salicarius112Streptococcus suitis223Streptococcus sitis112Streptococcus salicarius112Streptococcus suitis221Streptococcus situans111Streptococcus luteus111Streptococcus intermedius111Streptococcus intermedius111Streptococcus intermedius111Streptococcus intermedius111Strepto	Staphylococcus aureus	99				99
Enterococcus faecalis         33         33           Staphylococcus epidermidis         25         25           Staphylococcus nonlyticus         14         4         18           Streptococcus anginosus         10         4         14           Streptococcus agalactiae         10         10         10           Staphylococcus hominis         8         1         9           Staphylococcus cus hominis         7         7         7           Staphylococcus cus hominis         7         7         7           Staphylococcus cus hominis         7         7         7           Staphylococcus captrae         5         2         7           Enterococcus casseliflavus         4         3         3           Streptococcus cus setuitis         2         2         2           Enterococcus avium         2         2         2           Streptococcus constellatus         2         2         2           Streptococcus aditorius         1         2         2           Streptococcus salivarius         1         1         2           Streptococcus simulans         1         1         2           Streptococcus simulans         1	Enterococcus faecium	63				63
Staphylococcus epidermidis       25       25         Staphylococcus haemolyticus       14       4       18         Streptococcus aginosus       10       4       14         Streptococcus aginosus       10       4       10         Staphylococcus aginosus       10       4       10         Staphylococcus aginosus       7       9       9         Staphylococcus capitis       7       7       7         Staphylococcus caprae       5       2       7         Enterococcus casseliflavus       4       4       4         Staphylococcus acasseliflavus       4       4       3         Streptococcus acasseliflavus       4       4       3         Streptococcus acasseliflavus       4       3       3         Streptococcus acasseliflavus       4       3       3         Streptococcus pyogenes       2       2       2         Enterococcus affinosus       2       2       2       2         Streptococcus antiis       2       2       2       3       3         Streptococcus simulans       1       1       2       3       3       3       3       3       3       3 <t< td=""><td>Enterococcus faecalis</td><td>33</td><td></td><td></td><td></td><td>33</td></t<>	Enterococcus faecalis	33				33
Staphylococcus haemolyticus14418Streptococcus anginosus10414Streptococcus anginosus1010Staphylococcus hominis819Staphylococcus lugdunensis77Staphylococcus capitis77Staphylococcus caprae527Enterococcus casseliflavus44Staphylococcus caprae527Enterococcus asseliflavus44Staphylococcus pyogenes22Enterococcus avium22Streptococcus constellatus22Streptococcus alivarius11222Streptococcus salivarius11Streptococcus simulans11Micrococcus luteus11Streptococcus gallanarum11Streptococcus gallanarum11Streptococcus gallanarum11Streptococcus simulans11Micrococcus gallinarum11Streptococcus gallinarum1	Staphylococcus epidermidis	25				25
Streptococcus anginosus10414Streptococcus agalactiae1010Staphylococcus hominis81Staphylococcus lugdunensis7Staphylococcus capitis7Staphylococcus capitis7Staphylococcus capitis7Staphylococcus capitis7Staphylococcus capitis7Staphylococcus capitis7Staphylococcus capitis7Staphylococcus capitis2Enterococcus casseliflavus4Staphylococcus pyogenes2Enterococcus avium2Streptococcus constellatus2Streptococcus raffinosus2Streptococcus salivarius112Streptococcus simulans1Micrococcus luteus111Streptococcus gallinarum1Streptococcus gallinarum1Streptococcus parasanguinis1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Staphylococcus haemolyticus	14		4		18
Streptococcus agalactae         10         10           Staphylococcus hominis         8         1         9           Staphylococcus lugdunensis         7         7           Staphylococcus capitis         7         7           Staphylococcus capitis         7         7           Staphylococcus capitis         7         7           Staphylococcus caprae         5         2         7           Enterococcus caseliflavus         4         4         3           Streptococcus progenes         2         2         2           Enterococcus avium         2         2         2           Streptococcus constellatus         2         2         2           Streptococcus antitis         2         2         2           Streptococcus salivarius         1         1         2           Streptococcus salivarius         1         1         2           Streptococcus simulans         1         1         2           Streptococcus luteus         1         1         1           Micrococcus luteus         1         1         1           Streptococcus gallinarum         1         1         1           Streptococcus gallinar	Streptococcus anginosus	10		4		14
Staphylococcus homms819Staphylococcus lugdunensis77Staphylococcus capitis7Staphylococcus capitis7Staphylococcus caprae52Enterococcus caseliflavus4Staphylococcus pettenkoferi3Streptococcus poygenes2Enterococcus constellatus2Streptococcus constellatus2Streptococcus raffinosus2Streptococcus avium2Streptococcus avium2Streptococcus avium2Streptococcus salivarius112Streptococcus salivarius112Streptococcus salivarius112Streptococcus salivarius111Streptococcus gallinarum1Streptococcus gallinarum1Streptococcus prasanguinis1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Streptococcus agalactiae	10				10
Staphylococcus lugdunensis77Staphylococcus capitis77Staphylococcus capitis7Staphylococcus caprae52Enterococcus casseliflavus4Staphylococcus pettenkoferi3Streptococcus poygenes2Enterococcus constellatus2Streptococcus constellatus2Streptococcus constellatus2Streptococcus avium2Streptococcus avium2Streptococcus salivarius112Streptococcus salivarius112Streptococcus salivarius112Streptococcus salivarius111Streptococcus salivarius111Streptococcus galinarum1Streptococcus galinarum1Streptococcus paraanguinis1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Staphylococcus hominis	8		1		9
Staphylococcus capitis77Staphylococcus capita527Enterococcus casselifavus44Staphylococcus pettenkoferi33Streptococcus pyogenes22Enterococcus avium22Streptococcus constellatus22Streptococcus constellatus22Streptococcus raffinosus22Streptococcus salivarius112Streptococcus salivarius1122Streptococcus simulans11Micrococus luteus11Streptococcus gallinarum11Streptococcus gallinarum11Streptococcus predictiae11No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)Staply(0.0%)319 (100%)319 (100%)	Staphylococcus lugdunensis	7				7
Staphylococcus caprae527Enterococcus casseliflavus44Staphylococcus pettenkoferi3Streptococcus pyogenes2Enterococcus avium2Enterococcus constellatus2Streptococcus constellatus2Streptococcus raffinosus2Streptococcus raffinosus2Streptococcus salivarius112Streptococcus salivarius112Streptococcus simulans112Streptococcus luteus111Streptococcus gallinarum1Streptococcus premedius1Streptococcus presentina1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Staphylococcus capitis	7		•		7
Enterococcus casselfutors44Staphylococcus pettenkoferi33Streptococcus pyogenes22Enterococcus avium22Streptococcus constellatus22Enterococcus raffinosus22Streptococcus raffinosus22Streptococcus salivarius11222Streptococcus salivarius112312Streptococcus simulans11Micrococcus luteus11Streptococcus gallivarium11Streptococcus gallivarium11Streptococcus simulans11Streptococcus gallivarium11Streptococcus gallivarium11Streptococcus gallivarium11No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Staphylococcus caprae	5		2		1
Staphylococcus pertenkoperi33Streptococcus pyogenes22Enterococcus avium2Streptococcus constellatus2Enterococcus raffinosus2Streptococcus raffinosus2Streptococcus salivarius112Streptococcus salivarius112Streptococcus simulans1Micrococcus luteus111Streptococcus gallicarium1Streptococcus gallicarium1Streptococcus premedius1Streptococcus parasanguinis1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Enterococcus casselifiavus	4				4
Streptococcus pyogenes2Enterococcus avium2Streptococcus avium2Streptococcus constellatus2Enterococcus raffinosus2Streptococcus mitis2Streptococcus mitis2Streptococcus salivarius112Streptococcus simulans1Micrococcus luteus1Streptococcus gallinarum1Streptococcus gallinarum1Streptococcus parasanguinis1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Stupnylococcus pettenkoferi	3				3
Enterococcus uotum22Streptococcus constellatus22Enterococcus raffinosus22Streptococcus mitis22Streptococcus salivarius11Streptococcus salivarius11Streptococcus simulans11Micrococcus luteus11Streptococcus gallinarum11Streptococcus gallinarum11Streptococcus parasanguinis11No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Streptococcus pyogenes	2				2
Shephococcus constraints22Enterococcus raffinosus22Streptococcus mitis22Streptococcus salivarius1122Streptococcus simulans11Micrococcus luteus11Streptococcus gallinarum1Streptococcus gallinarum1Streptococcus intermedius1Streptococcus parasanguinis1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Enterococcus uotum	2				2
Enterococcus najinosus22Streptococcus mitis22Streptococcus salivarius1122Streptococcus sinulans112Streptococcus dysgalactiae111Streptococcus gallinarum111Streptococcus gallinarum1Streptococcus parasanguinis1No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Enterococcus constellutus	2				2
Streptococcus mins2Streptococcus salivarius112Streptococcus pneumoniae112Staphylococcus simulans111Micrococcus luteus111Streptococcus gallinarum11Streptococcus intermedius11Streptococcus parasanguinis11No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Strentococcus rujjinosus	2				2
Streptococcus suitorius112Streptococcus pneumoniae112Staphylococcus simulans11Micrococcus luteus11Streptococcus dysgalactiae11Enterococcus gallinarum11Streptococcus intermedius11Streptococcus parasanguinis11No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)	Streptococcus salizarius	2		1		2
Shreptococcus pincunoniae112Staphylococcus simulans11Micrococcus luteus11Streptococcus dysgalactiae11Enterococcus gallinarum11Streptococcus intermedius11Streptococcus parasanguinis11No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)	Streptococcus suitourius	1	1	1		2
Micrococcus similarity11Micrococcus luteus11Streptococcus gysgalactiae11Enterococcus gallinarum11Streptococcus intermedius11Streptococcus parasanguinis11No. of subtotal (%)306 (95.9%)1 (0.3%)12 (3.8%)0 (0.0%)319 (100%)	Stanhulococcus simulans	1	1			∠ 1
Streptococcus dusgalactiae       1         Image: Streptococcus gallinarum       1         Streptococcus intermedius       1         Streptococcus parasanguinis       1         No. of subtotal (%)       306 (95.9%)       1 (0.3%)       12 (3.8%)       0 (0.0%)       319 (100%)	Micrococcus luteus	1				1
Enterococcus gallinarum         1         1           Enterococcus gallinarum         1         1           Streptococcus intermedius         1         1           Streptococcus garasanguinis         1         1           No. of subtotal (%)         306 (95.9%)         1 (0.3%)         12 (3.8%)         0 (0.0%)         319 (100%)	Strentococcus duscalactiae	1				1
Streptococcus intermedius         1         1           Streptococcus parasanguinis         1         1           No. of subtotal (%)         306 (95.9%)         1 (0.3%)         12 (3.8%)         0 (0.0%)         319 (100%)	Enterococcus gallinarum	1				1
Streptococcus parasanguinis         1         1           No. of subtotal (%)         306 (95.9%)         1 (0.3%)         12 (3.8%)         0 (0.0%)         319 (100%)	Strentococcus intermedius	1				1
No. of subtotal (%) 306 (95.9%) 1 (0.3%) 12 (3.8%) 0 (0.0%) 319 (100%)	Streptococcus narasanouinis	1				1
	No. of subtotal (%)	306 (95.9%)	1 (0.3%)	12 (3.8%)	0 (0.0%)	319 (100%)

# Table 1. Comparison of the results of the Bruker Biotyper and ASTA MicroIDSys systems.

<u>Caracian</u>	Bruker $\geq$ 2.0		Bruker 1.7≤, <2.0		
Species	$ASTA \ge 140$	ASTA < 140	$ASTA \ge 140$	ASTA < 140	Total
Other bacteria					
Corynebacterium striatum	25				25
Člostridium difficile	9		1		10
Corynebacterium jeikeium	1			1	2
Clostridium hathewayi	1				1
Bacillus cereus	1				1
Actinomyces odontolyticus	1				1
Bacillus circulans	1				1
Corynebacterium			1		1
tuberculostearicum			1		1
No. of subtotal (%)	39 (92.9%)	0 (0.0%)	2 (4.8%)	1 (2.4%)	42 (100%)
Candida spp. and other					
fungi †					
Candida tropicalis	41		1		42
Candida albicans	35		1		36
Candida glabrata	18		1		19
Candida parapsilosis	6		1		7
Trichosporon asahii	2				2
Cryptococcus neoformans				1	1
No. of subtotal (%)	102 (95.3%)	0 (0.0%)	4 (3.7%)	1 (0.9%)	107 (100%)
No. of total (%)	840 (96.7%)	2 (0.2%)	24 (2.8%)	3 (0.3%)	869 (100%)

#### Table 1. Cont.

\* When any result was below the cutoff score (<140 for MicroIDSys and <2.0 for Bruker Biotyper), we performed 16S rRNA gene sequencing for bacterial identification. The results of the *16S rRNA* gene sequencing are provided in parentheses for the cases whose results by molecular testing were different from those by MALDI–TOF MS (<sup>1</sup> *E. fergusonii*; <sup>2</sup> *A. soli*; <sup>3</sup> *A. calcoaceticus*; <sup>4</sup> *B. nasdae*). <sup>†</sup> Molecular testing was not performed for fungal isolates.

The correct identification rate at the species level was evaluated for the results of 792 bacterial isolates, as 16S rRNA sequencing could not be performed for 108 fungal isolates and 14 bacterial isolates because of the lack of samples. The correct identification rate with a score above the target cutoff ( $\geq$ 2.0, Bruker Biotyper, and  $\geq$ 140 for MicroIDSys and) was 94.2% (746/792) by Bruker Biotyper and 95.7% (758/792) by ASTA MicroIDSys (p = 0.177).

Minor errors were defined as misidentification at the species level with a score above the target cutoff ( $\geq$ 2.0 for Bruker Biotyper and  $\geq$  140 for MicroIDSys). The minor error rate was 1.1% (9/792) for Bruker Biotyper and 1.6% (13/792) for ASTA MicroIDSys (p = 0.388). Major errors were defined as misidentification at the genus level ( $\geq$ 140 for MicroIDSys and  $\geq$  1.7 for Bruker Biotyper). Exceptionally, the misidentification of *S. aureus* at the species level was considered as a major error because of the clinical importance of this species. The major error rate was 0.3% (2/792) for both Bruker Biotyper and ASTA MicroIDSys, and there was no case of misidentification of *S. aureus* as other coagulase-negative staphylococci. The isolate of *Weisella cibaria* was misidentified as *E. coli*, and the isolate of *Brevibacterium frigoritolerans* as *Lactobacillus jensenii* by Bruker Biotyper. The isolate of *Staphylococcus warneri* was misidentified as *Azotobacter nigricans* and the isolate of *Kluyvera ascorbata* was misidentified as *Raoultella ornithinolytica* by ASTA MicroIDSys.

The two systems showed discrepant results for 31 isolates (3.4%). However, at the genus level, they were in agreement, except for five isolates. The identification results for the 16S rRNA gene sequencing for these isolates are shown in Table 2. The 16S rRNA sequencing could not be performed for 14 bacterial isolates due to the lack of samples and one fungal isolate. Among the 16 results for the 16S rRNA sequencing, nine and three were in agreement with those of Bruker Biotyper and ASTA MicroIDSys, respectively (p = 0.066).

	Bruker		ASTA		Identification by	
	Identification	Score	Identification	Score	16S rRNA Sequencing (Accession)	
Concordant at genus level	Klebsiella variicola	1.984	Klebsiella pneumoniae	203	Klebsiella variicola (CP010523.2)	
	Klebsiella variicola	2.111	Klebsiella pneumoniae	177	Klebsiella variicola (CP010523.2)	
	Klebsiella variicola	2.264	Klebsiella pneumoniae	236	N/T	
	Klebsiella variicola	2.242	Klebsiella pneumoniae	144	N/T	
	Klebsiella variicola	1.903	Klebsiella pneumoniae	157	N/T	
	Streptococcus pneumoniae	2.216	Streptococcus mitis	194	Streptococcus pneumoniae (LN831051.1)	
	Streptococcus pneumoniae	2.117	Streptococcus mitis	176	Streptococcus pneumoniae (NR_028665.1)	
	Streptococcus pneumoniae	2.144	Streptococcus mitis	169	Streptococcus mitis (NR_028665.1)	
	Streptococcus pneumoniae	1.894	Streptococcus sobrinus	111	Streptococcus mitis (NR_028664.1)	
	Streptococcus vestibularis	2.149	Streptococcus salivarius	223	Streptococcus salivarius (CP009913.1)	
	Streptococcus infantis	1.884	Streptococcus mitis	169	Streptococcus infantis (LC096227.1)	
	Streptococcus oralis	2.056	Streptococcus mitis	160	N/T	
	Enterobacter asburiae	2.111	Enterobacter cloacae	207	Enterobacter kobei (CP017181.1)	
	Enterobacter asburiae	2.151	Enterobacter cloacae	206	N/T	
	Enterobacter kobei	2.263	Enterobacter cloacae	207	N/T	
	Citrobacter youngae	2.108	Citrobacter freundii	179	Citrobacter braakii (NR_028687.1)	
	Citrobacter koseri	2.291	Citrobacter amalonaticus	226	N/T	
	Paenibacillus urinalis	2.127	Paenibacillus macerans	112	Paenibacillus urinalis (NR_044178.1)	
	Paenibacillus urinalis	2.247	Paenibacillus lactis	153	Paenibacillus urinalis (NR_044178.1)	
	Paenibacillus barengoltzii	2.167	Paenibacillus macerans	171	Paenibacillus barengoltzii (NR_113988.1)	
	Paenibacillus glucanolyticus	1.931	Paenibacillus ginsengagri	196	N/T	
Ps	Pseudomonas monteilii	2.084	Pseudomonas putida	181	N/T	
	Burkholderia lata	2.155	Burkholderia cepacia	198	N/T	
	Corynebacterium simulans	2.237	Corynebacterium striatum	151	N/T	
	Providencia rettgeri	1.833	Providencia stuartii	168	N/T	
	Candida metapsilosis	1.708	Candida orthopsilosis	134	N/T	
Discordant at the genus level	Kluyvera ascorbata	2.128	Raoultella ornithinolytica	167	Kluyvera ascorbata (NR_028677.1)	
level	Escherichia coli	2.059	Weissella confusa	239	Weissella cibaria (LC096236.1)	
	Staphylococcus warneri	1.970	Azotobacter nigricans	143	(NR_025922.1)	
	Streptococcus pneumoniae	2.073	Saccharomyces cerevisiae	120	N/T	
	Clostridium difficile	1.961	Eggerthella lenta	171	N/T	

 Table 2. List of isolates with discrepant results by the Bruker Biotyper and ASTA MicroIDSys systems.

The Bruker Biotyper showed invalid results for 10 isolates (1.1%), and ASTA MicroIDSys showed invalid results for four isolates (0.4%) (p = 0.178). The identification results for the 16S rRNA gene sequencing for these isolates are shown in Table 3.

	Bruker		ASTA		Identification by
	Identification	Score	Identification	Score	<sup>–</sup> 16S rRNA Sequencing (Accession)
Correct identification by Bruker *	Pantoea calida	2.236	Invalid Identifie	cation	Pantoea calida (AB907785.1)
ey braner	Weeksella virosa	2.184	Invalid Identification		Weeksella virosa (CP002455.1)
	Streptococcus mitis	1.805	Invalid Identification		Streptococcus mitis (NR_028664.1)
Correct identification by ASTA *	Invalid Identif	fication	Propionibacterium acnes	200	Propionibacterium acnes (CP003084.1)
-)	Invalid Identif	fication	Moraxella osloensis	160	Moraxella osloensis (CP014234.1)
	Invalid Identification Invalid Identification Invalid Identification		Weissella confusa	203	Weissela cibaria (LC096236.1)
			Brevibacillus centrosporus 134		Brevibacillus limnophilus (NR_024822.1)
			Paenibacillus lactis	115	Paenibacillus spp. (JN377815.1)
Incorrect identification	Lactobacillus jensenii	2.003	Invalid Identifie	cation	Brevibacterium frigoritolerans (NR_117474.1)
	Invalid Identif	fication	Staphylococcus arlettae	117	Pseudoglutamicibacter cumminsii (NR_044895.1)
	Invalid Identif	fication	Parvimonas micra	125	Dermabacter vaginalis (CP012117.1)
	Invalid Identification		Bacillus simplex	131	Brevibacterium frigoritolerans (NR_117474.1)
	Invalid Identification		Knoellia subterranea	132	Janibacter hoylei (NR_104794.1)
	Invalid Identification		Paenibacillus timonensis	125	Lysinibacillus spp. (HE586367.1)

**Table 3.** List of isolates whose identification showed invalid results by the Bruker Biotyper or ASTA

 MicroIDSys systems.

\* It was regarded as correct identification when the results by Bruker or ASTA was concordant with the results by 16S rRNA sequencing at the genus level.

## 4. Discussion

In this study, we evaluated the performance of ASTA MicroIDSys, a newly developed MALDI–TOF system which can be routinely used in clinical microbiology laboratories, in comparison with Bruker Biotyper which is widely used. Most of the bacteria and yeasts which are commonly isolated in clinical laboratories were correctly identified by MALDI–TOF MS, while a few uncommon bacteria, including *Brevibacterium* spp., *Pseudog-lutamicibacter* spp., and *Janibacter* spp., were given invalid results.

The performance of MicroIDSys was comparable to that of Bruker Biotyper with the overall concordance rate of 91.9%. This result is in the same line with a previous study which reported good agreement of results between Bruker Biotyper and ASTA MicroIDSys. In that study, identical results with confidence scores ( $\geq$ 2.0 for Bruker Biotyper) and acceptable scores ( $\geq$ 140 for ASTA MicroIDSys) were obtained for 86.1% from the 4919 isolates recovered from sputum, urine, and pus samples [8]. In another study which evaluated the performance of ASTA MicroIDSys compared to that of VITEK MS, the ASTA MicroIDSys correctly identified 96.7% of isolates to species level which was comparable to VITEK MS (97.3%) [9].

As microorganisms are identified by MALDI–TOF MS systems, using prerecorded protein spectra, which are mostly based on ribosomal proteins, MALDI–TOF MS systems are intrinsically limited to differentiate closely related species of *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Raoultella* [10,11]. The discrepant results between Bruker Biotyper and ASTA MicroIDSys in this study also revealed the known limitation of MALDI–TOF MS relies heavily on the database containing the spectra of known organisms. It is critical that it includes a sufficient number of isolates for each species, grown under a variety of conditions such that the spectral library for the organism is sufficiently robust to account for the inherent variability expected for any organism. Most of the discrepant or invalid results in this study were from microorganisms that are not frequently isolated in clinical laboratories. Of note, the ASTA MicroIDSys misidentified the rare microorganisms with scores over 140. There should be caution when interpreting the results of ASTA MicroIDSys with microorganisms such as *Paenibacillus* spp. or *Weissella* spp.

Molecular approaches could be useful for correct identification. There has been a study which applied whole genome-based bacterial identification system for clinical isolates that were not identified with MALDI–TOF MS systems [12]. It evaluated thirtysix isolates including *Corynebacterium* spp., *Brevibacterium* spp., and *Brevundimonas* spp. which were also not correctly identified by MALDI–TOF MS in our study. Genome-based identification may be an additional tool in the future. However, whole genome sequencing is yet burdensome in cost and methodology for clinical microbiology laboratories. Targeted sequencing of 16S rRNA, *gyrB*, or *rpoB* for bacteria, and internal transcribed spacer (ITS) or 28S region for yeasts can be the practical approach when MALDI–TOF MS cannot give the correct identification.

Except for the above mentioned studies, there are other studies that evaluated the performance of ASTA MicroIDSys on yeast [13], anaerobic bacteria [14], mycobacteria [15], and filamentous fungi [16]. As the aim of this study was to evaluate the utilization of ASTA MicroIDSys for routine identification in clinical microbiology laboratories, there was no selection on the types of microorganisms or on the types of samples. This study evaluated the performance of ASTA MicroIDSys by using microorganisms isolated from all types of samples including blood specimen at a 750-bed general hospital.

There are several limitations in this study. First, when the results obtained with the Bruker Biotyper and the ASTA MicroIDSys were identical at the species with scores above the target cutoff, we considered the results as correct identification without performing16S rRNA sequencing. Second, there is inborn limitation of 16S rRNA sequencing as it may show poor discrimination power for some genera in Gram-positive cocci [17], *Enterobacteriaceae* [18], or for *Campylobacter* spp. [19]. However, the BLAST alignment of 16S rRNA sequencing showed only one type of species with  $\geq$ 99% homology for all the clinical isolates in this study. Third, 16S rRNA sequencing could not be performed for 14 bacterial isolates which showed discrepant results by the Bruker Biotyper and ASTA MicroIDSys due to lack of samples. Lastly, molecular testing for fungal isolates could not be performed due to our laboratory setting, which might limit the exact evaluation of performance for identification of fungi. However, as 100 (95.2%) among the 105 isolates of *Candida* spp. showed concordant results at the species level by the Bruker Biotyper and the ASTA MicroIDSys with scores above the target cutoff, it was demonstrated that ASTA MicroIDSys could reliably identify clinically important fungal isolates.

# 5. Conclusions

Since the introduction of MALDI–TOF MS in clinical microbiology laboratories, it has been widely used for microbial identification because of its speed and accuracy. In this study, ASTA MicroIDSys showed reliable performance on microbial identification that was comparable to that of the Bruker Biotyper. Therefore, ASTA MicroIDSys could be applied for identification of microorganism in clinical microbiology laboratories.

**Author Contributions:** Conceptualization, J.-S.K.; data curation, Y.C. and M.H.; funding acquisition, J.-S.K.; methodology, J.-S.K.; writing—original draft, Y.C.; writing—review and editing, M.H. and J.-S.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare (grant number: HI17C2067) and by Nosquest (Seongnam, Korea). The funders had no role in study design; data collection and interpretation; or the decision to submit the work for publication.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the institutional review board of Kangdong Sacred Heart Hospital (IRB file No. Kangdong NON2018-001, 18 September 2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

# Conflicts of Interest: The authors declare no conflict of interest.

# References

- Seng, P.; Drancourt, M.; Gouriet, F.; La Scola, B.; Fournier, P.E.; Rolain, J.M.; Raoult, D. Ongoing revolution in bacteriology: Routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin. Infect. Dis.* 2009, 49, 543–551. [CrossRef] [PubMed]
- 2. Claydon, M.A.; Davey, S.N.; Edwards-Jones, V.; Gordon, D.B. The rapid identification of intact microorganisms using mass spectrometry. *Nat. Biotechnol.* **1996**, *14*, 1584–1586. [CrossRef] [PubMed]
- Martiny, D.; Busson, L.; Wybo, I.; El Haj, R.A.; Dediste, A.; Vandenberg, O. Comparison of the Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J. Clin. Microbiol.* 2012, 50, 1313–1325. [CrossRef] [PubMed]
- 4. Richter, S.S.; Sercia, L.; Branda, J.A.; Burnham, C.A.; Bythrow, M.; Ferraro, M.J.; Garner, O.B.; Ginocchio, C.C.; Jennemann, R.; Lewinski, M.A.; et al. Identification of Enterobacteriaceae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system. *Eur. J. Clin. Microbiol. Infect. Dis.* **2013**, *32*, 1571–1578. [CrossRef] [PubMed]
- 5. Bessede, E.; Angla-Gre, M.; Delagarde, Y.; Sep Hieng, S.; Menard, A.; Megraud, F. Matrix-assisted laser-desorption/ionization biotyper: Experience in the routine of a University hospital. *Clin. Microbiol. Infect.* **2011**, *17*, 533–538. [CrossRef] [PubMed]
- Kassim, A.; Pfluger, V.; Premji, Z.; Daubenberger, C.; Revathi, G. Comparison of biomarker based Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) and conventional methods in the identification of clinically relevant bacteria and yeast. *BMC Microbiol.* 2017, 17, 128. [CrossRef] [PubMed]
- Wattal, C.; Oberoi, J.K.; Goel, N.; Raveendran, R.; Khanna, S. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) for rapid identification of micro-organisms in the routine clinical microbiology laboratory. *Eur. J. Clin. Microbiol. Infect. Dis.* 2017, 36, 807–812. [CrossRef] [PubMed]
- Lee, Y.; Sung, J.Y.; Kim, H.; Yong, D.; Lee, K. Comparison of a New Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Platform, ASTA MicroIDSys, With Bruker Biotyper for Species Identification. *Ann. Lab. Med.* 2017, 37, 531–535. [CrossRef] [PubMed]
- Jung, J.; Kim, S.Y.; Park, Y.J.; Lee, J.; Suk, H.S.; Ha, S.I.; Shin, J.S.; Park, K.G.; Kim, Y. Comparison of the ASTA MicroIDSys and VITEK MS matrix-assisted laser desorption/ionization time-of-flight mass spectrometry systems for identification of clinical bacteria and yeasts. J. Infect. Chemother. 2020, 26, 1328–1333. [CrossRef] [PubMed]
- Bilecen, K.; Yaman, G.; Ciftci, U.; Laleli, Y.R. Performances and Reliability of Bruker Microflex LT and VITEK MS MALDI-TOF Mass Spectrometry Systems for the Identification of Clinical Microorganisms. *BioMed. Res. Int.* 2015, 2015, 516410. [CrossRef] [PubMed]
- Wang, H.; Fan, Y.Y.; Kudinha, T.; Xu, Z.P.; Xiao, M.; Zhang, L.; Fan, X.; Kong, F.; Xu, Y.C. A Comprehensive Evaluation of the Bruker Biotyper MS and Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Systems for Identification of Yeasts, Part of the National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) Study, 2012 to 2013. J. Clin. Microbiol. 2016, 54, 1376–1380. [CrossRef] [PubMed]
- Ha, S.M.; Kim, C.K.; Roh, J.; Byun, J.H.; Yang, S.J.; Choi, S.B.; Chun, J.; Yong, D. Application of the Whole Genome-Based Bacterial Identification System, TrueBac ID, Using Clinical Isolates That Were Not Identified With Three Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) Systems. *Ann. Lab. Med.* 2019, *39*, 530–536. [CrossRef] [PubMed]
- Lee, H.; Park, J.H.; Oh, J.; Cho, S.; Koo, J.; Park, I.C.; Kim, J.; Park, S.; Choi, J.S.; Shin, S.Y.; et al. Evaluation of a new matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for the identification of yeast isolation. *J. Clin. Lab. Anal.* 2019, 33, e22685. [CrossRef] [PubMed]
- Kim, D.; Ji, S.; Kim, J.R.; Kim, M.; Byun, J.H.; Yum, J.H.; Yong, D.; Lee, K. Performance evaluation of a new matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, ASTA MicroIDSys system, in bacterial identification against clinical isolates of anaerobic bacteria. *Anaerobe* 2020, *61*, 102131. [CrossRef] [PubMed]
- Yoo, I.Y.; Shim, H.J.; Yun, S.A.; Kang, O.K.; Chung, Y.N.; Kim, T.Y.; Lee, H.; Kim, J.; Park, Y.J.; Huh, H.J.; et al. Evaluation of the ASTA MicroIDSys matrix-assisted laser desorption ionization time-of-flight mass spectrometry system for identification of mycobacteria directly from positive MGIT liquid cultures. *Int. J. Infect. Dis.* 2021, 102, 172–177. [CrossRef]
- Lee, H.; Oh, J.; Sung, G.H.; Koo, J.; Lee, M.H.; Lee, H.J.; Cho, S.I.; Choi, J.S.; Park, Y.J.; Shin, J.H.; et al. Multilaboratory Evaluation of the MALDI-TOF Mass Spectrometry System, MicroIDSys Elite, for the Identification of Medically Important Filamentous Fungi. *Mycopathologia* 2021, *186*, 15–26. [CrossRef]
- 17. Mignard, S.; Flandrois, J.P. 16S rRNA sequencing in routine bacterial identification: A 30-month experiment. *J. Microbiol. Methods* **2006**, *67*, 574–581. [CrossRef] [PubMed]
- 18. Mollet, C.; Drancourt, M.; Raoult, D. rpoB sequence analysis as a novel basis for bacterial identification. *Mol. Microbiol.* **1997**, *26*, 1005–1011. [CrossRef] [PubMed]
- 19. Gorkiewicz, G.; Feierl, G.; Schober, C.; Dieber, F.; Köfer, J.; Zechner, R.; Zechner, E.L. Species-specific identification of campylobacters by partial 16S rRNA gene sequencing. *J. Clin. Microbiol.* **2003**, *41*, 2537–2546. [CrossRef] [PubMed]