1   **Performance of the Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight**

2   **Mass Spectrometry System for Rapid Bacterial Identification in Two Diagnostic Centers in**

3   **China.**

4   Running Title: Vitek MS performance in China

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22

23   ABSTRACT

24   Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

25   systems had not been officially launched for diagnostic use in clinical microbiology

26   laboratories in China until 2012. Here, we reported the findings from the first large-scale

27   evaluation study of Vitek MS for routine bacterial identification in two major diagnostic

28   centers in Beijing and Hong Kong. A total of 2,266 unique isolates, representing 56 genera

29   and 127 species were analyzed, and results were compared to those obtained by Vitek 2.

30   Any discrepancies were resolved by 16S rRNA sequencing. Overall, Vitek MS provided

31   correct identification for 2,246 (99.1%) isolates, including 2,193 (96.8%) isolates with correct

32   species-level identifications and 53 (2.3%) isolates matched at genus level only. Vitek MS

33   surpassed Vitek 2 consistently in species-level identification for the important pathogens,

34   including non-*Enterobacteriaceae* Gram-negative bacilli (94.7%vs92%), Staphylococci

35   (99.7%vs92.4%), Streptococci (92.6%vs79.4%), Enterococci (98.8%vs92.6%), and Clostridia

36   (97.3%vs55.5). The findings demonstrated that the Vitek MS is highly accurate and reliable

37   for routine bacterial identification in clinical settings in China.

38   Keywords: MALDI-TOF MS, Vitek MS, bacterial identification

1. INTRODUCTION

In an era of dramatic increase of drug resistance, rapid bacterial identification facilitates better management of antimicrobial therapies and infection control. Nowadays, semi-automated biochemical test platforms such as Vitek 2 (BioMerieux, Marcy l'Etoile, France), or PHOENIX (BD Diagnostics, Sparks, MD, USA) are commonly used in clinical microbiology laboratories to complement the conventional cultured based method for routine bacterial identification, reducing the average time-to-identification to about 10 hours (Chatzigeorgiou *et al.* 2011). However, the reagent cost is extremely high in the laboratory settings in densely populated countries, such as China.

The drawback can be potentially addressed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which was originally applied in biochemical industries for analysis of different macromolecules. The concept of microbial identification by measuring the unique bacterial proteomic fingerprints was firstly proposed about 30 years ago (Anhalt & Fenselau 1975). With the advent of automated algorithm in mass spectral acquisition and database-matching, bacterial identification can be performed in batchwise with an average reagent cost of less than 1 US dollar and the turnaround time of only a few minutes per sample. Numerous studies have described and compared the performance of two most common MALDI-TOF MS systems, the Bruker Biotyper (Bruker Daltonics, Germany) and the Vitek MS (BioMerieux, Marcy l'Etoile, France), in identification of aerobic bacteria, anaerobes, yeasts and mycobacteria isolated from primary cultures (Chen *et al.* 2013; Garner *et al.* 2013; Manji *et al.* 2013; Rychert *et al.* 2013; Westblade *et al.* 2013). The results consistently showed that MALDI-TOF MS offered equivalent or even superior accuracy in comparison to conventional phenotypic methods. Nevertheless, despite this promising data reported elsewhere, MALDI-TOF MS had not been officially launched for clinical use in China until the bioMerieux Vitek MS system was approved by the

China State Food and Drug Administration (SFDA) for in *vitro* diagnostic (IVD) purpose in 2012.

In the present study, we report the findings of a large scale evaluation of the Vitek MS IVD system for the identification of aerobic and anaerobic bacteria in two major diagnostic centers in Beijing and Hong Kong, two major cosmopolitan cities with good geographic representation of northern and southern China respectively. To the best of our knowledge, no similar studies of this sample size have been performed in developing countries including China.

2. MATERIALS AND METHODS

2.1 Study sites. The performance of the Vitek MS IVD system was evaluated at the Departments of Microbiology in Chinese People's Liberation Army General Hospital (PLA 301 Hospital) of Beijing and Queen Mary hospital (QMH) of Hong Kong. PLA 301 Hospital, which serves patients mostly from the North China regions, is the biggest military tertiary hospital in China with 3,500-beds and around 100,000 inpatients per year, whereas QMH is a tertiary referral university-affiliated acute hospital with 1,600 beds, serving a population of 0.53 million resident in southern area of China.

Prior to the initiation of the study, the operators in the two different study sites were trained by the same team of technical staff from BioMerieux so that the techniques used in Vitek MS target slide preparation, instrument operation and data interpretation were consistent with each other.

2.2 Bacterial isolates. During a three-month period (March 2013 – May 2013), all aerobic and anaerobic bacterial isolates recovered from various clinical specimens, such as blood,

urine, stool, cerebrospinal fluid, wound swabs, throat swabs, sputum and other lower respiratory specimens, were collected for this study prospectively.

Prior to MALDI-TOF MS analysis, bacterial isolates were recovered on appropriate agar media (Columbia horse blood agar for aerobes or facultative anaerobes and neomycin blood agar supplemented with hemin and vitamin K for anaerobes) under 35℃ incubation for 24h to 72h in aerobic, microaerophilic or anaerobic conditions as appropriate. Test results of the isolates from the same patients were deduplicated to ensure that a particular isolate was only tested once.

2.3 Conventional bacterial identification. In each diagnostic centre, after the Gram staining and determination of catalase and oxidase activities, bacterial identification relied on semi-automated biochemical test platforms, Vitek 2, using the GP, GN, NH or ANC cards (BioMerieux, Marcy l'Etoile, France) according to manufacturer's instructions. The criterion used for the acceptance of species-level identifications obtained from Vitek 2 was that the isolates were identified as the only choice with a confidence value of ≥85%. Genus-level identifications were reported when 2 to 3 species of the same genus were given. A no identification (No ID) result was denoted as (i) single identification result with confidence value less than 85%, (ii) multiple identification results including species of different genera or (iii) an "no ID" flag was provided by Vitek 2 owing to no identification present in the system database.

2.4 Vitek MS identification. Isolates were identified by Vitek MS system using a single deposit directly from bacterial colonies without any prior extraction step according to the manufacturer's guideline. In brief, a portion of a fresh colony was applied onto an individual spot of the 48-wells Vitek MS-DS disposable target slide, followed by overlaying with 1µl ready-to-use Vitek MS Matrix solution — saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid (BioMerieux, Marcy l'Etoile, France).

After drying, the target plate was loaded onto the Vitek MS mass spectrometer for target

interrogation. The system reported the best identification match(es) along with confidence

value(s) from 0% to 99.9%.

Samples returned with single identification results with any confidence values were

considered as highly confidence at species level, whereas the result was only considered as

genus level identification when the system proposed a split identification with any

confidence values (low discrimination) that included species of the same genus. A "No ID"

result was denoted if (i) no identification result was provided by the system or (ii) split

identification result including species of different genera were given.

Samples with "No ID" were retested with a single deposit and the repeated result was

considered as the final result for that specimen.

In addition to the *Escherichia coli* ATCC 8739, which served as the calibration control and

internal identification control, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa*

ATCC 10145 and *Clostridium perfringens* ATCC 13124 were used as the external positive

control for Gram positive, Gram negative and anaerobic bacteria respectively. For quality

control purpose, these external positive controls and the bacteria-free Vitek MS Matrix

solution, which served as a negative control, were analyzed on each day of testing.

2.5 Final reference identification and Discrepancy resolution. When the Vitek MS system

proposed a species-level identification that was completely matched with that provided by

Vitek 2, the result was considered as the final reference identification and no further

investigation was performed.

In case species-level identification was not available from either systems, or if there was

mismatch between the two systems, bacterial DNA was extracted and mailed to Hong Kong

for 16S rRNA sequencing. The resulting sequence was run through the Ridom and

136   16SpathDB databases to determine the final reference identification (Janda & Abbott 2007;

137   Woo *et al.* 2011).

138   A correct identification was defined as any result from Vitek MS and Vitek 2 concordant with

139   the final reference identification at the species and/or genus level, whereas a

140   misidentification (mis-ID) result was denoted when the bacterial identifications obtained

141   from Vitek MS and Vitek 2 did not match with the final reference bacterial identification.

142   **2.6 Statistical analysis**. Pearson's Chi-square test or Fisher's Exact test, where appropriate,

143   were used to compare the results obtained by Vitek MS and Vitek II with the same specimen.

144   Cohen's kappa coefficients (κ) were also calculated to determine the level of agreement

145   between two methods.

146

147 **3. RESULTS**

148 **3.1 Overall results**. A total of 2,266 bacterial isolates were analyzed in this study. This

149 included 1,581 aerobic Gram negative bacteria, 535 aerobic Gram positive bacteria and 150

150 anaerobes, representing 56 genera and 127 species. Overall, Vitek MS correctly identified

151 99.1% (2246/2266) of the isolates, including 96.8% (2193/2266) to the species level and an

152 additional 2.3% (53/2266) to the genus level. The remaining 0.9% (20/2266) isolates were

153 either misidentified (n=4) or denoted as no identification (n=16).

154 **3.2 Aerobic Gram-Negative Bacteria.** The 1,581 aerobic Gram negative isolates collected in

155 the study were categorized into the *Enterobacteriaceae* family (n=1,182) and non-

156 *Enterobacteriaceae* Gram negative organisms (n=399) (Table S1).

157 Vitek MS results agreed with the reference final identification for 99.4% (1175/1182) of the

158 *Enterobacteriaceae* isolates, with 97.1% (1148/1182) identified to species level and 2.3%

159 (28/1182) corrected to the genus level only (Table S1). Correct species-level identification

160 was consistently obtained for all isolates among 12 species (*Enterobacter aerogenes*,

161 *Enterobacter gergoviae*, *Enterobacter sakazakii*, *E. coli*, *Escherichia hermannii*, *Klebsiella*

162 *oxytoca*, *Morganella morganii*, *Providencia rettgeri*, *Providencia stuartii*, *Serratia*

163 *liquefaciens*, *Serratia marcescens* and *Salmonella typhi*) whereas four species were more

164 likely to have a correct genus-level ID rather than species level by using Vitek MS *: Proteus*

165 *vulgaris* (n=17, 0% species, 100% genus), *Raoultella ornithinolytica* (n=4, 0% species, 75%

166 genus, 25% no ID), *Salmonella enterica ssp arizonae* (n=1, 0% species, 100% genus) and

167 *Salmonella paratyphi A* (n=1, 0% species, 100% genus).

168 Vitek MS misidentified four isolates (two *Shigella spp.* isolates were misidentified as *E. coli*,

169 one *Raoultella ornithinolytica* was misidentified as *Enterobacter aerogenes* and one *Proteus*

170 *mirabilis* was misidentified as *Proteus vulgaris / Proteus penneri*) and failed to identify three

171 isolates ("no ID" for one each of *Citrobacter freundii* complex, *Citrobacter koseri* and

172 *Klebsiella pneumoniae*).

173 Vitek MS correctly reported 99.2% (396/399) of non-*Enterobacteriaceae* Gram negative

174 organisms, which encompassed 38 species and 25 genera, to species level (94.7%, 378/399)

175 or to genus but not species level (4.5%, 18/399) (Table S1). A total of 29 species of

176 organisms, including those commonly encountered important pathogens, such as

177 *Acinetobacter baumannii* complex (n=44)*, Pseudomonas aeruginosa* (n=212) and

178 *Stenotrophomonas maltophilia* (n=67), showed concordant species-level identification with

179 the reference method for over 90% of their isolates. Some species, including *Aeromonas*

180 *caviae* (n=4), *Burkholderia pseudomallei* (n=2), *Ochrobacturm intermedium* (n=2),

181 *Pseudomonas alcaligenes* (n=1) and *Pseudomonas otitidis* (n=1), were only limited to genus-

182 level identification for all the isolates (0% species, 100% genus). The three isolates that

183 could not be identified with Vitek MS (i.e. "No ID" flaggings) included one each of

184 *Acinetobacter radioresistens*, *Campylobacter coli* and *Pasteurella dagmatis.*

185 **3.3 Aerobic Gram-positive Bacteria**. Among the 535 Gram-positive bacterial isolates (Table

186 S2), the Staphylococci (n=357), Streptococci (n=68) and Enterococci (n=81) are the most

187 commonly encountered pathogens in the clinical laboratories. In addition, 29 isolates of

188 other Gram-positive cocci (n=9) and Gram-positive rods (n=20) were also collected during

189 the study period (Table S2).

190 Of the 357 staphylococci, all but one was correctly identified to species level by Vitek MS.

191 The missing isolate was *Staphylococcus lugdunensis*, which was only corrected to genus level

192 as multiple choices of species-level identification were given. Similarly, with the exception of

193 one *Enterococcus durans* isolate, which was returned with "No ID" from the system, Vitek

194 MS provided correct species-level identification for all the Enterococci. The species-level

195 identification for the Streptococci appeared to be more challenging than Staphylococci and

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196   Enterococci, with 63/68 (92.6%) of the isolates being correctly identified to the species level.

197   In particular, only 75% of the *Streptococcus bovis* group, 77.8% of the *Streptococcus*

198   *dysgalactiae* and 85.7% of the *Streptococcus anginosus* group were correctly identified to

199   species level (Table S2). Nevertheless, the Vitek MS correctly differentiated *Streptococcus*

200   *pneumoniae* from *Streptococcus mitis* group for all but one case. The final reference

201   identification for this case was *S. pneumoniae*, the Vitek MS, however, reported a split

202   identification between *S. pneumoniae* and *S. mitis* group.

203   The remaining 29 Gram-positive organisms represented 9 additional genera. Vitek MS can

204   readily provide 28 (96.6%) accurate identifications, with 27 (93.1%) corrected to species-

205   level. Only one isolate of *Nocardia brasiliensis* was reported as no identification (Table S2).

206   **3.4 Anaerobes**. A total of 150 anaerobic bacterial isolates (8 genera and 19 species) were

207   analyzed in this study. The Vitek MS provided correct identification for 95.3% (143/150) of

208   the isolates, including 94.0% (141/150) down to species-level and 1.3% (2/150) achieved

209   genus-level. The remaining 4.7% (7/150) isolates were provided with "no ID" results by the

210   system (Table S3).

211   Vitek MS correctly identified 100% (28/28) and 97.3% (107/110) of the *Bacteroides spp.* and

212   *Clostridia* spp., respectively. All the *Clostridium difficile* (n=85) and the 17 *Clostridium*

213   *perfringens* (n=17) isolates were correctly identified to species level by Vitek MS. However,

214   only 6 correct species-level identifications were obtained from those 12 non-Clostridia and

215   non-Bacteroides anaerobes (Table S3).

216   **3.5   Vitek MS versus Vitek 2.** The Vitek 2 system provided 2,237 (98.7%) correct

217   identification to the species level (n=2096; 92.5%) and genus level (n=141; 6.2%)

218   respectively. Comparison of performance in species-level identification between Vitek MS

219   and Vitek 2 were summarized in Table 1. In brief, with the exception of *Enterobacteriaceae*,

220 non-Enterobacteriaceae Gram-negative organisms and *Bacteroides*, for which species-level

221 identifications were performed equally well by both system, Vitek MS surpassed the

222 performance of Vitek 2 for those clinically important pathogens, including the Staphylococci,

223 Streptococci, Enterococci and Clostridia. Conversely, Vitek 2 system demonstrated better

224 performance in identifying the species of those non-Clostridia and non-Bacteroides

225 anaerobes.

226 The final reference identifications for the 20 organisms with "mis ID" and "no ID" obtained

227 from Vitek MS were listed in Table 2.

228

229 **4 DISCUSSION**

230 MALDI-TOF MS systems have been demonstrated to be a fast, accurate and reliable

231 technique for identification of clinical relevant bacteria in many countries. This was the first

232 large-scale evaluation study of Vitek MS performed in China. The major strength of this

233 study is the extensive breadth of tested clinical isolates from good geographic

234 representation of northern and southern region of China.

235 In this study, an almost perfect agreement between the identifications inferred by Vitek MS

236 and those provided by phenotypic and genotypic reference methods was observed, with the

237 overall concordance of 99.1%, which is better than those reported in Switzerland (94.7%)

238 (Benagli *et al.* 2011), Netherlands (95.1%) (van Veen *et al.* 2010), France (96.2%) (Dubois *et*

239 *al.* 2012).

240 Among the isolates collected during the study period, more than 50% belonged to the

241 *Enterobacteriaceae* family. In accordance with a previous study that evaluated Vitek MS

242 performance for identification of *Enterobacteriaceae* (Richter *et al.* 2013), our study showed

243 accurate genus- and species-level identifications for majority of isolates (99.4 %) with only a

244 small number of misidentifications. There was no particular members of *Enterobacteriaceae*

245 for which the Vitek MS consistently failed except *Shigella*. It has been well-documented that

246 *Shigella* spp. and *E. coli* are indistinguishable from each other using 16S rRNA sequencing or

247 MALDI-TOF MS systems, which is attributed to the taxonomic similarity of these two

248 organisms (Johnson 2000; van den Beld & Reubsaet 2012). The misidentification, however,

249 is considered as a major drawback from clinical point of view, particularly for diagnosis of

250 acute gastrointestinal infections that necessitates to be resolved by conventional

251 biochemical tests, such as lactose fermentation and indole production or motility.

252 Richter *et al* reported the inability of Vitek MS to discriminate the members of *Citrobacter*,

253 such as *C. freundii*, *C. youngae*, *C. braakii* and *C. werkmanii*, and also the members of

254 *Enterobacter*, such as *E. asburiae*, *E. cloacae*, *E. hormaechei*, and *E. kobei* (Richter, Sercia et

255 al. 2013). Fortunately, as the clinical significances and the drug susceptibilities among the

256 members are similar, the impact of misidentification by Vitek MS on patient care is minimal

257 (Janda *et al.* 1994; Paauw *et al.* 2008). In our study, these species were interpreted as

258 "*Citrobacter freundii* complex" and "*Enterobacter cloacae* complex" with the accuracy of

259 89.3% and 97.4% respectively, showing that this is probably the best approach to report

260 MALDI-TOF MS results for these strains.

261 For non-*Enterobacteriaceae* Gram-negative bacilli, our study showed that the Vitek MS IVD

262 system showed correct genus and species identification for 99.2% of the tested isolates. The

263 result was in consistent with a multicenter evaluation study performed in US (Manji,

264 Bythrow et al. 2013). Manji *et al* evaluated the performance of Vitek MS v2.0 for

265 identification of 558 unique non-*Enterobacteriaceae* Gram-negative bacilli in US and was

266 returned with overall accurate species and genus identification of 90.9% (Manji, Bythrow et

267 al. 2013). Likewise, Van Veen *et al*, using the Microflex LT instrument, showed that the

268 system could achieve the genus and species identification to 94.3% with similar variety of

269 tested strains in Europe (van Veen, Claas et al. 2010). It should be noted that incorrect

identification of non-*Enterobacteriaceae* Gram-negative bacilli always results in significant negative clinical impact, especially for species with high drug resistant rate, such as *Acinetobacter baumannii* complex, or for bacteria with limited therapeutic choices, such as *Stenotrophomonas maltophilia* (Dortet *et al.* 2006; Howard *et al.* 2012; Vila & Pachon 2012). No false identification was obtained in this study, indicating that Vitek MS is a reliable diagnostic tool for enhancing the management of infections associated with non-*Enterobacteriaceae* Gram-negative bacilli in China.

In clinical microbiology laboratories, distinguishing one species from another among some Gram positive bacteria, especially the coagulase-negative Staphylococci, the viridians group Streptococci and some Enterococci, is often unreliable and overly complicated using conventional biochemical methods (Ruoff 2011). It has been previously demonstrated that MALDI-TOF MS system provides accurate and reliable platform for identification of these Gram-positive aerobic bacteria (Moon *et al.* 2013; Rychert, Burnham et al. 2013). Consistent with the findings of those studies, we also experienced the significant better performance of Vitek MS than Vitek 2 in species identification of Gram-positive bacteria, especially Staphylococci (99.7%), Enterococci (98.8%) and Streptococci (92.6%). This is particularly important for diagnosis of sepsis and meningitis, in which identification to species level is necessary. Additionally, the Vitek MS was shown to readily differentiate *S. pneumoniae* from *S. mitis* group species in our study. This was recognized as a strength of Vitek MS over MALDI Biotyper given that misidentifications of *S. mitis* group strains as *S. pneumoniae* by MALDI Biotyper were reported in several studies (Bizzini *et al.* 2010; Cherkaoui *et al.* 2010; Neville *et al.* 2011; McElvania Tekippe *et al.* 2013). However, this problem appears to have been overcome in the latest release of the Bruker Biotyper software (version 3.1; MBT-BDAL-5627 MSP library, Bruker Daltonics) (Harju *et al*. 2014).

294   Anaerobic bacteria represent a group of major infectious agents identified in clinical

295   microbiology laboratories. However, owing to the low doubling time and relatively inert

296   biochemical reactivity, phenotypic identification methods, including the commercially

297   available biochemical kits, can be both time-consuming and laborious, hindering the

298   selection of appropriate therapy. The development of MALDI-TOF MS systems provides the

299   opportunity for rapid and accurate identification of anaerobe (Nagy *et al.* 2009; Garner,

300   Mochon et al. 2013). Importantly, Vitek MS correctly identified all of the 85 tested *C. difficile*

301   isolates to species level, which facilitate the timely initiation of appropriate infection control

302   measures to limit the spread of disease. Nevertheless, MALDI-TOF MS showed varied rates

303   of identification for other anaerobes. Asides from *Clostridia* and *Bacteroides*, Vitek MS only

304   correctly identified 2/3 of anaerobes to genus or species level. The finding was similar to

305   other previous investigations, which only resulted in up to 61% correct species-level

306   identifications for similar variety of strains (Seng *et al.* 2009; La Scola *et al.* 2011). This

307   highlights the importance of extending the coverage of database with the mass spectra of

308   additional reference strains to improve the identification capacity for anaerobes using

309   MALDI-TOF MS. Nevertheless, since there were only a small number of isolates were

310   analyzed for several rarely encountered species, the study findings may underestimate the

311   system's true capabilities in identification of anaerobes.

312   In conclusion, Vitek MS offered equivalent or even superior accuracy in comparison to Vitek

313   2 in identification of bacterial species among different clinically important pathogens. The

314   introduction of Vitek MS system in our clinical laboratories would therefore facilitate

315   shorter turnaround time with improved diagnostic accuracy for routine bacterial

316   identification. With the current mass spectral reference database, Vitek MS is accurate and

317   reliable for identification of nearly all aerobic bacteria, *Clostridia spp.* and *Bacteroides spp.*

318   in our region. However, this technology still cannot completely supersede the biochemical

319　test panels, which are essential to supplement the areas of weakness of Vitek MS, such as

320　the inability to differentiate *Shigella* species from *E. coli* and the suboptimal performance in

321　identification of non-Clostridia and non-Bacteroides anaerobic bacteria. On the other hand,

322　continuous expansion of Vitek MS spectral database is needed, particularly for anaerobic

323　bacteria, in order to improve both the efficiency and the accuracy of Vitek MS in routine

324　diagnostic microbiology.

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437 of medically important bacteria by 16S rRNA gene sequencing using a novel comprehensive
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Table S1: Identification results from Gram-negative aerobic bacteria obtained from Vitek MS and Vitek 2.

Reference identification (no. of isolates)	Vitek MS No. (%) of isolates with correct ID to the level of				Vitek 2 No. (%) of isolates with correct ID to the level of			
	Species[*]	Genus[†]	No ID[‡]	Mis ID[§]	Species	Genus	No ID	Mis ID
Enterobacteriaceae (1,182)	**1,148 (97.1)**	**27 (2.3)**	**3 (0.3)**	**4 (0.3)**	**1,156 (97.8)**	**26 (2.2)**	**0**	**0**
Citrobacter freundii complex[‖] (28)	25 (89.3)	2 (7.1)	1 (3.6)	0	28 (100)	0	0	0
Citrobacter koseri (11)	10 (91)	0	1 (9)	0	11 (100)	0	0	0
Enterobacter aerogenes (35)	35 (100)	0	0	0	35 (100)	0	0	0
Enterobacter cloacae complex[¶] (76)	74 (97.4)	2 (2.6)	0	0	74 (97.4)	2 (2.6)	0	0
Enterobacter gergoviae (1)	1 (100)	0	0	0	1 (100)	0	0	0
Enterobacter sakazakii (2)	2 (100)	0	0	0	2 (100)	0	0	0
Escherichia coli (595)	595 (100)	0	0	0	595 (100)	0	0	0
Escherichia hermannii (1)	1 (100)	0	0	0	1 (100)	0	0	0
Klebsiella oxytoca (48)	48 (100)	0	0	0	48 (100)	0	0	0
Klebsiella pneumoniae (135)	134 (99.3)	1 (0.7)	0	0	134 (99.3)	1 (0.7)	0	0
Klebsiella ozaenae (1)	0	0	1 (100)	0	1 (100)	0	0	0
Morganella morganii (24)	24 (100)	0	0	0	24 (100)	0	0	0
Proteus mirabilis (146)	145 (99.3)	0	0	1 (0.7)	146 (100)	0	0	0
Proteus vulgaris (17)	0	17 (100)	0	0	0	17 (100)	0	0
Providencia rettgeri (3)	3 (100)	0	0	0	3 (100)	0	0	0
Providencia stuartii (3)	3 (100)	0	0	0	3 (100)	0	0	0
Raoultella ornithinolytica (4)	0	3 (75)	0	1 (25)	0	4 (100)	0	0
Serratia liquefaciens (1)	1 (100)	0	0	0	1 (100)	0	0	0
Serratia marcescens (46)	46 (100)	0	0	0	46 (100)	0	0	0
Salmonella enterica ssp arizonae (1)	0	1 (100)	0	0	1 (100)	0	0	0
Salmonella paratyphi A (1)	0	1 (100)	0	0	1 (100)	0	0	0
Salmonella typhi (1)	1 (100)	0	0	0	1 (100)	0	0	0
Shigella flexneri (1)	0	0	0	1 (100)	0	1 (100)	0	0
Shigella sonneii (1)	0	0	0	1 (100)	0	1 (100)	0	0
Non-*Enterobacteriaceae* Gram-Negative organisms (399)	**378 (94.7)**	**18 (4.5)**	**3 (0.8)**	**0**	**367 (92.0)**	**20 (5.0)**	**12 (3.0)**	**0**
Achromobacter xylosoxidans (5)	5 (100)	0	0	0	5 (100)	0	0	0
Acinetobacter baumannii complex (44)	40 (91.0)	4 (9.0)	0	0	42 (95.4)	1 (2.3)	1 (2.3)	0
Acinetobacter jonnsonii (1)	1 (100)	0	0	0	0	0	1 (100)	0
Acinetobacter radioresistens (1)	0	0	1 (100)	0	1 (100)	0	0	0
Acinetobacter ursingii (3)	3 (100)	0	0	0	3 (100)	0	0	0
Actinobacillus ureae (1)	1 (100)	0	0	0	0	0	1 (100)	
Aeromonas caviae (4)	0	4 (100)	0	0	0	4 (100)	0	0
Bergeyella zoohelcum (1)	1 (100)	0	0	0	0	0	1 (100)	0
Bordetella pertussis (1)	1 (100)	0	0	0	0	0	1 (100)	0
Burkholderia cepacia complex[#] (4)	4 (100)	0	0	0	4 (100)	0	0	0
Burkholderia pseudomallei (2)	0	2 (100)	0	0	2 (100)	0	0	0
Campylobacter jejuni (2)	2 (100)	0	0	0	0	0	2 (100)	0
Campylobacter coli (1)	0	0	1 (100)	0	0	0	1 (100)	0
Chromobacterium violaceum (1)	1 (100)	0	0	0	1 (100)	0	0	0
Chryseobacterium indologenes (4)	4 (100)	0	0	0	4 (100)	0	0	0
Cupriavidus pauculus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Delftia acidovorans (3)	3 (100)	0	0	0	1 (33.3)	0	2 (66.6)	0
Eikenella corrodens (1)	1 (100)	0	0	0	1 (100)	0	0	0
Elizabethkingia meningoseptica (3)	3 (100)	0	0	0	3 (100)	0	0	0
Haemophilus influenzae (2)	2 (100)	0	0	0	2 (100)	0	0	0
Haemophilus parahaemolyticus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Moraxella osloensis (1)	1 (100)	0	0	0	0	0	1 (100)	0
Neisseria gonorrhoeae (2)	2 (100)	0	0	0	1 (50)	1 (50)	0	0
Ochrobacturm intermedium (2)	0	2 (100)	0	0	0	1 (50)	1 (50)	0
Pasteurella dagmatis (1)	0	0	1 (100)	0	0	1 (100)	0	0
Pasteurella multocida (4)	4 (100)	0	0	0	0	4 (100)	0	0
Plesiomonas shigelloides (1)	1 (100)	0	0	0	1 (100)	0	0	0
Pseudomonas aeruginosa (212)	209 (98.6)	3 (1.4)	0	0	209 (98.6)	3 (1,4)	0	0
Pseudomonas alcaligenes (1)	0	1 (100)	0	0	0	1 (100)	0	0
Pseudomonas otitidis (1)	0	1 (100)	0	0	0	1 (100)	0	0
Pseudomonas putida (8)	8 (100)	0	0	0	6 (75)	2 (25)	0	0
Ralstonia mannitolilytica (2)	1 (50)	1 (50)	0	0	2 (100)	0	0	0
Ralstonia pickettii (1)	1 (100)	0	0	0	1 (100)	0	0	0
Shewanella algae (6)	6 (100)	0	0	0	6 (100)	0	0	0
Stenotrophomonas maltophilia (67)	67 (100)	0	0	0	66 (98.5)	1 (1.5)	0	0
Vibrio cholerae (1)	1 (100)	0	0	0	1 (100)	0	0	0
Vibrio parahaemolyticus (2)	2 (100)	0	0	0	2 (100)	0	0	0
Vibrio vulnificus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Total (1,581)	**1,526 (96.6)**	**45 (2.8)**	**6 (0.4)**	**4 (0.2)**	**1,523 (96.3)**	**46 (2.9)**	**12 (0.8)**	**0**

[*] Species-level identification was denoted when only one identification result was obtained from Vitek MS.
[†] Genus-level identification was denoted when a split identification (low discrimination) that included species of the same genus was obtained from Vitek MS.
[‡] No ID was denoted when (i) multiple identifications that included species of different genera, or (ii) "no ID" flagging was obtained from Vitek MS.
[§] Mis-ID was denoted when the bacterial identifications obtained from Vitek MS did not match with the final reference bacterial identification.
[‖] *Citrobacter freundii complex includes Citrobacter freundii, C. braakii, C. gilenii, C. murliniae, C. werkmanii and youngae.*
[¶] *Enterobacter cloacae complex includes Enterobacter cloacae, Enterobacter anigenus, Enterobacter intermedium and Enterobacter kobei.*
[#] *Burkholderia cepacia complex includes B. cepacia, B. multivorans, B. stabilis, B. vietnamiensis, B. ambifaria, B. anthina, B. pyrrocinia and genomovar III and VI.*

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Table S2: Identification results from Gram-positive aerobic bacteria obtained from Vitek MS and Vitek 2.

Reference identification (no. of isolates)	Vitek MS				Vitek 2			
	No. (%) of isolates with correct ID to the level of				No. (%) of isolates with correct ID to the level of			
	Species[*]	Genus[†]	No ID[‡]	Mis ID[§]	Species	Genus	No ID	Mis ID
***Staphylococcus* (357)**	**356 (99.7)**	**1 (0.3)**	**0**	**0**	**330 (92.4)**	**26 (7.3)**	**0**	**1 (0.3)**
Staphylococcus aureus (213)	213 (100)	0	0	0	195 (91.5)	18 (18.5)	0	0
Staphylococcus caprae (1)	1 (100)	0	0	0	1 (100)	0	0	0
Staphylococcus capitis (12)	12 (100)	0	0	0	12 (100)	0	0	0
Staphylococcus cohnii (3)	3 (100)	0	0	0	3 (100)	0	0	0
Staphylococcus epidermidis (55)	55 (100)	0	0	0	54 (98.2)	1 (1.8)	0	0
Staphylococcus haemolyticus (16)	16 (100)	0	0	0	15 (93.8)	1 (6.2)	0	0
Staphylococcus hominis (41)	41 (100)	0	0	0	37 (90.2)	4 (9.8)	0	0
Staphylococcus lugdunensis (9)	8 (88.9)	1 (11.1)	0	0	8 (88.9)	0	0	1 (11.1)
Staphylococcus saprophyticus (3)	3 (100)	0	0	0	2 (66.7)	1 (33.3)	0	0
Staphylococcus sciuri (2)	2 (100)	0	0	0	2 (100)	0	0	0
Staphylococcus simulans (1)	1 (100)	0	0	0	1 (100)	0	0	0
Staphylococcus schleiferi (1)	1 (100)	0	0	0	0	1 (100)	0	0
***Enterococcus* (81)**	**80 (98.8)**	**0**	**1 (1.2)**	**0**	**75 (92.6)**	**4 (4.9)**	**0**	**2 (2.5)**
Enterococcus avium (4)	4 (100)	0	0	0	4 (100)	0	0	0
Enterococcus casseliflavus (4)	4 (100)	0	0	0	3 (75)	1 (25)	0	0
Enterococcus faecalis (27)	27 (100)	0	0	0	27 (100)	0	0	0
Enterococcus faecium (38)	38 (100)	0	0	0	35 (92.1)	2 (5.3)	0	1 (2.6)
Enterococcus gallinarum (4)	4 (100)	0	0	0	3 (75)	1 (25)	0	0
Enterococcus durans (4)	3 (75)	0	1 (25)	0	3 (75)	0	0	1 (25)
***Streptococcus* (68)**	**63 (92.6)**	**4 (5.9)**	**1 (1.5)**	**0**	**54 (79.3)**	**10 (14.7)**	**2 (3)**	**2 (3)**
Streptococcus agalactiae (12)	12 (100)	0	0	0	10 (83.3)	2 (16.7)	0	0
Streptococcus bovis group[||] (4)	3 (75)	0	1 (25)	0	3 (75)	1 (25)	0	0
Streptococcus dysgalactiae (9)	7 (77.8)	2 (22.2)	0	0	9 (100)	0	0	0
Streptococcus anginosus group[¶] (7)	6 (85.7)	1 (14.3)	0	0	3 (42.9)	3 (42.9)	0	1 (14.2)
Steptococcus gallolyticus (1)	1 (100)	0	0	0	1 (100)	0	0	0
Steptococcus gordonii (1)	1 (100)	0	0	0	1 (100)	0	0	0
Streptococcus mitis group[#] (11)	11 (100)	0	0	0	8 (72.7)	2 (18.2)	0	1 (9.1)
Steptococcus parasanguinis (1)	1 (100)	0	0	0	1 (100)	0	0	0
Streptococcus pneumoniae (10)	9 (90)	1 (10)	0	0	9 (90)	1 (10)	0	0
Streptococcus pyogenes (5)	5 (100)	0	0	0	5 (100)	0	0	0
Streptococcus salivarius (4)	4 (100)	0	0	0	3 (75)	1 (25)	0	0
Streptococcus sanguinis (1)	1 (100)	0	0	0	1 (100)	0	0	0
Streptococcus suis (2)	2 (100)	0	0	0	0	0	2 (100)	0
Other Gram-positive cocci (9)	**9 (100)**	**0**	**0**	**0**	**7 (77.8)**	**2 (22.2)**	**0**	**0**
Granulicatella adiacens (1)	1 (100)	0	0	0	1 (100)	0	0	0
Lactococcus garvieae (1)	1 (100)	0	0	0	0	1 (100)	0	0
Micrococcus luteus (6)	6 (100)	0	0	0	6 (100)	0	0	0
Pediococcus acidilactici (1)	1 (100)	0	0	0	0	1 (100)	0	0
Gram-positive rods (20)	**18 (90)**	**1 (5)**	**1 (5)**	**0**	**9 (45)**	**3 (15)**	**8 (40)**	**0**
Arcanobacterium haemolyticum (2)	2 (100)	0	0	0	2 (100)	0	0	0
Arcanobacterium pyogenes (1)	1 (100)	0	0	0	1 (100)	0	0	0
Bacillus cereus group[¤] (4)	4 (100)	0	0	0	0	0	4 (100)	0
Bacillus circulans (1)	1 (100)	0	0	0	0	0	1 (100)	0
Bacillus megaterium (1)	1 (100)	0	0	0	0	0	1 (100)	0
Corynebacterium jerkerium (1)	1 (100)	0	0	0	1 (100)	0	0	0
Corynebacterium striatum (3)	3 (100)	0	0	0	1 (33.3)	1 (33.3)	1 (33.3)	0
Corynebacterium urealyticum (2)	2 (100)	0	0	0	2 (100)	0	0	0
Listeria monocytogenes (3)	3 (100)	0	0	0	1 (33.3)	2 (66.7)	0	0
Listeria ivanovii (1)	0	1 (100)	0	0	1 (100)	0	0	0
Nocardia brasiliensis (1)	0	0	1 (100)	0	0	0	1 (100)	0
Total (535)	**526 (98.3)**	**6 (1.1)**	**3 (0.6)**	**0**	**475 (88.8)**	**45 (8.4)**	**10 (1.9)**	**5 (0.9)**

[*] Species-level identification was denoted when only one identification result was obtained from Vitek MS.

[†] Genus-level identification was denoted when a split identification (low discrimination) that included species of the same genus was obtained from Vitek MS.

[‡] No ID was denoted when (i) multiple identifications that included species of different genera, or (ii) "no ID" flagging was obtained from Vitek MS.

[§] Mis-ID was denoted when the bacterial identifications obtained from Vitek MS did not match with the final reference bacterial identification.

[||] *Streptococcus bovis group* includes *S. bovis, S. alactolyticus, S. infantarius* and *S. gallolyticus*.

[¶] *Streptococcus anginosus group* includes *S. anginosus, S. constellatus* and *S. intermedius*.

[#] *Streptococcus mitis group* includes *S. sanguis, S. parasanguis, S. gordonii, S. crista, S. oralis, S. mitis, S. peroris* and *S. infantis*.

[¤] *Bacillus cereus group* includes *Bacillus cereus, Bacillus thuringiensis* and *Bacillus mycoides*.

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Table S3: Identification results from anaerobic bacteria obtained from Vitek MS and Vitek 2.

Reference identification (no. of isolates)	Vitek MS				Vitek 2			
	No. (%) of isolates with correct ID to the level of				No. (%) of isolates with correct ID to the level of			
	Species[*]	Genus[†]	No ID[‡]	Mis ID[§]	Species	Genus	No ID	Mis-ID
Clostridia (110)	**107 (97.3)**	**0**	**3 (2.7)**	**0**	**61 (55.5)**	**49 (44.5)**	**0**	**0**
Clostridium bifermentans (2)	2 (100)	0	0	0	0	2 (100)	0	0
Clostridium butyricum (1)	1 (100)	0	0	0	0	1 (100)	0	0
Clostridium difficile (85)	85 (100)	0	0	0	42 (49.4)	43 (50.6)	0	0
Clostridium innocuum (2)	0	0	2 (100)	0	0	2 (100)	0	0
Clostridium novyi (1)	0	0	1 (100)	0	0	1 (100)	0	0
Clostridium perfringens (17)	17 (100)	0	0	0	17 (100)	0	0	0
Clostridium ramosum (1)	1 (100)	0	0	0	1 (100)	0	0	0
Clostridium septicum (1)	1 (100)	0	0	0	1 (100)	0	0	0
Bacteroides (28)	**28 (100)**	**0**	**0**	**0**	**28 (100)**	**0**	**0**	**0**
Bacteroides vulgatus (4)	4 (100)	0	0	0	4 (100)	0	0	0
Bacteroides thetaiotaomicron (3)	3 (100)	0	0	0	3 (100)	0	0	0
Bacteroides ovatus (6)	6 (100)	0	0	0	6 (100)	0	0	0
Bacteroides fragilis (15)	15 (100)	0	0	0	15 (100)	0	0	0
Other anaerobes (12)	**6 (50)**	**2 (16.7)**	**4 (33.3)**	**0**	**9 (75)**	**1 (8.3)**	**2 (16.7)**	**0**
Actinomyces europaeus (1)	1 (100)	0	0	0	0	0	1 (100)	0
Atopobium parvulum (1)	0	0	1 (100)	0	0	0	1 (100)	0
Eggerthella lenta (4)	0	2 (50)	2 (50)	0	4 (100)	0	0	0
Propionibacterium avidum (1)	0	0	1 (100)	0	0	1 (100)	0	0
Propionibacterium acnes (1)	1 (100)	0	0	0	1 (100)	0	0	0
Finegoldia magna (1)	1 (100)	0	0	0	1 (100)	0	0	0
Parvimonas micra (3)	3 (100)	0	0	0	3 (100)	0	0	0
Anaerobes (150)	**141 (94.0)**	**2 (1.3)**	**7 (4.7)**	**0**	**98 (65.4)**	**50 (33.3)**	**2 (1.3)**	**0**

[*] Species-level identification was denoted when only one identification result was obtained from Vitek MS.

[†] Genus-level identification was denoted when a split identification (low discrimination) that included species of the same genus was obtained from Vitek MS.

[‡] No ID was denoted when (i) multiple identifications that included species of different genera, or (ii) "no ID" flagging was obtained from Vitek MS.

[§] Mis-ID was denoted when the bacterial identifications obtained from Vitek MS did not match with the final reference bacterial identification.

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Table 1: Comparison of performance in species-level identification between Vitek MS and Vitek 2

Bacterial group	No. of isolates	Percentage (%) of species-level identification		kappa / *p* value
		Vitek MS	Vitek 2	
Enterobacteriaceae	1,182	97.1	97.8	0.863 / 0.598
Non-Enterobacteriaceae Gram-negative organism	399	94.7	92	0.484 / 0.074
Staphylococcus	357	99.7	92.4	0.066 / <0.01
Streptococcus	68	92.6	79.4	0.134 / <0.01
Enterococcus	81	98.8	92.6	0.270 / 0.043
Gram-positive rods	20	90	45	0.031 / <0.01
Clostridia	110	97.3	55.5	0.067 / <0.01
Bacteroides	28	100	100	n.a.[*]
Non-Clostridia and non-Bacteroides anaerobes	12	50	75	0.100 / 0.023
Anaerobes (Overall)	150	94	65.4	0.037 / 0.011

[*]Cohen's kappa coefficients (κ) cannot be calculated as the results from both methods are not variable.

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Table 2: Final reference identifications for the 20 isolates with mis ID or no ID obtained from Vitek MS

Final reference ID	No. of isolates	Identities given by Vitek MS (Confidence value)	Identities given by Vitek 2 (Confidence value)	Identities given by 16S rRNA
Citrobacter freundii complex	1	No ID	*Citrobacter braakii (97%)*	*Citrobacter murliniae*
Citrobacter koseri	1	No ID	*Citrobacter koseri (99%)*	*Citrobacter koseri*
Klebsiella ozaenae[*]	1	No ID	no ID	*Klebsiella ozaenae*
Proteus mirabilis	1	*Proteus penneri* (50.0%); *Proteus vulgaris* (50.0%)	*Proteus mirabilis* (99%)	*Proteus mirabilis*
Raoultella ornithinolytica	1	*Enterobacter aerogenes* (99.9%)	*Raoultella ornithinolytica (99%); Raoultella planticola (97%)*	*Raoultella ornithinolytica*
Shigella flexneri	1	*E. coli* (99.9%)	*Shigella flexneri (99.9%)*	*Shigella flexneri*
Shigella sonneii	1	*E. coli* (99.9%)	*Shigella sonneii (99.9%)*	*Shigella sonneii*
Acinetobacter radioresistens	1	No ID	*Acinetobacter radioresistens(99.9%)*	*Acinetobacter radioresistens*
Campylobacter coli	1	No ID	no ID	*Campylobacter coli*
Pasteurella dagmatis	1	No ID	*Pasteurella dagmatis* (99%); *Pasteurella stomatis*(97%)	*Pasteurella dagmatis*
Streptococcus bovis group	1	No ID	*Streptococcus bovis (96%); Streptococcus equinus(91%)*	*Streptococcus bovis/Streptococcus gallolyticus*
Enterococcus durans	1	No ID	*Enterococcus gallinarum* (88%)	*Enterococcus durans*
Nocardia brasiliensis[*]	1	No ID	No ID	*Nocardia brasiliensis*
Clostridium innocuum[*]	2	No ID	No ID	*Clostridium innocuum*
Clostridium novyi[*]	1	No ID	No ID	*Clostridium novyi*
Atopobium parvulum[*]	1	No ID	No ID	*Atopobium parvulum*
Eggerthella lenta	2	No ID	*Eggerthella lenta*	*Eggerthella lenta*
Propionibacterium avidum	1	No ID	*Propionibacterium granulosum* (91%); *Propionibacterium propionicus* (88%)	*Propionibacterium avidum*

[*] *Species absent from database*

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