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Role of Matrix-Assisted Laser Desorption Ionization–Time-of-Flight Mass Spectrometry for Species Identification of *Acinetobacter* Strains

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Abstract

Introduction *Acinetobacter* species has become a leading cause of nosocomial infections in recent years.

Objectives The aim of the study was to establish the usefulness of matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry (MS) for the identification of *Acinetobacter* species with respect to conventional biochemical methods and MicroScan WalkAway 96 Plus system and to compare the antibiotic susceptibility test results Kirby–Bauer disk diffusion method with MicroScan WalkAway 96 Plus automated identification and antimicrobial susceptibility testing system.

Materials and Methods The study sample comprised 100 clinical isolates of *Acinetobacter* species. They were all identified using MALDI-TOF MS and compared with other two identification systems.

Statistical Analysis Comparison of categorical variables by Fisher's exact test or Pearson's chi-square test was done. All statistical tools were two tailed, and a significant level $p < 0.05$ was used. All statistical tests were performed using SPSS v22.0 (Armonk IBM Corp., New York, United States). Cohen's kappa coefficients were also calculated and used as applicable.

Results MALDI-TOF MS revealed 92 *A. baumannii*, 2 *Acinetobacter nosocomialis*, 3 *Acinetobacter lwoffii*, and 1 each was identified as *Acinetobacter junii*, *Acinetobacter johnsonii*, and *Acinetobacter tandoii*. There was moderate agreement between identification by MicroScan WalkAway and MALDI-TOF, and substantial agreement between conventional biochemical tests and MALDI-TOF. We found that there was a 100% categorical agreement with respect to susceptibility of aminoglycosides (amikacin, gentamicin, tobramycin) and cephalosporins (ceftazidime, cefepime, cefotaxime) between disk diffusion method and MicroScan WalkAway 96 Plus system. Total of 16 errors were observed.

Conclusion Although MALDI-TOF MS could be useful to identify *A. baumannii* but not other species in the genus, it is a rapid, reliable method and can be routinely used in clinical laboratories.

Keywords
- *Acinetobacter* species
- antibiotic sensitivity
- MALDI-TOF
- MicroScan

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Introduction

According to most recent scientific literature, *Acinetobacter* species are the second most common nonfermenting gram-negative pathogens isolated from clinical samples after *Pseudomonas aeruginosa*.[1] The genus *Acinetobacter* comprises 30 named species and 9 genomic species. Consequently, more relevant species have been grouped together as *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex (ABC) (*A. baumannii*, *A. calcoaceticus*, *Acinetobacter* genospecies 3 *and A.* genospecies 13TU).[2,3] However, there are other species that may also have clinical significance. They cause wide range of infections such as bacteremia, pneumonia, skin and soft tissue infections, and urinary tract infections (UTIs). Non-*baumannii Acinetobacter* species are emerging pathogens and have also been isolated from patients with bacteremia, endocarditis, and meningitis.[4–7]

The currently available phenotypic identification systems cannot precisely distinguish *Acinetobacter* species. The conventional biochemical methods (CBMs) are tedious and time consuming and still only vaguely divide into groups and are not able to speciate the organisms. Automated phenotypic systems have been increasingly used in many clinical laboratories for identification of the bacteria and susceptibility testing. These systems decrease the labor and time required when compared with that for conventional phenotypic methods. Some of the commonly used automated systems are MicroScan WalkAway 96 Plus, BD Phoenix, and VITEK 2. These systems accurately identify few species but have failed to discriminate within the *A. calcoaceticus*–ABC.[8]

Matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry (MS) measures highly abundant (ribosomal) proteins found in all microorganisms, and the characteristic patterns obtained from these proteins are matched to a library to identify an organism reliably and accurately. This method is simple, fast, and cost-effective, requiring only small amounts of samples. However, nonfermenting gram-negative bacilli, including *Acinetobacter* species, may be misidentified because of an incomplete database.[9]

Several genotypic methods have been developed to differentiate *Acinetobacter* species. The 16S ribosomal RNA and RNA polymerase β-subunit (rpoB) gene sequencing approaches have been widely used. There is an abundance of rpoB polymorphisms in these *Acinetobacter* species; hence, it was suggested to be fairly accurate method for the identification of *Acinetobacter* species.[10,11] However, these molecular techniques are unsuitable for routine identification of *Acinetobacter* species, as they are laborious, expensive, and time consuming.[12]

Very few Indian studies are available regarding the evaluation of MALDI-TOF MS to identify *Acinetobacter* species. Therefore, the purpose of the present study was to assess the ability of MALDI-TOF MS to identify *Acinetobacter* species in a tertiary care center in India.

Materials and Methods

A cross-sectional study was conducted in the department of microbiology at a tertiary care teaching hospital located in Rishikesh, Uttarakhand, after the approval of the Institutional Ethics Committee (letter no. AIIMS/IEC/20/258).

The objectives of this study were as follows:

1. To identify *Acinetobacter* up to species level using MALDI-TOF MS.
2. To evaluate MALDI-TOF MS in comparison with automated phenotypic methods.
3. To compare the antibiotic susceptibility test results Kirby–Bauer disk diffusion method and MicroScan WalkAway 96 Plus automated identification and antimicrobial susceptibility testing (ID/AST) system.

Inclusion Criteria

The inclusion criteria are nonlactose fermenting, oxidase negative, nonmotile, gram-negative coccobacilli, isolated from various clinical samples such as blood, urine, pus, cerebrospinal fluid, central venous catheter tip, tissue, respiratory samples, body fluids obtained from outpatient/inpatient department patients.

Exclusion Criteria

The exclusion criteria are clinical samples such as urine catheter tip, intercostal drainage tip, tracheal aspirate/swab after removing the tracheostomy tube-lactose fermenting gram-negative bacilli, nonlactose fermenting, oxidase positive gram-negative bacteria, gram-positive cocci.

Study Duration and Sample Size

Hundred *Acinetobacter* spp. isolates obtained from various clinical samples received in Bacteriology Laboratory of AIIMS, Rishikesh from December 2018 to March 2020 (18 months) were included in the study.

All test isolates obtained from various clinical samples were subjected to conventional biochemical tests, MALDI-TOF MS and MicroScan WalkAway 96 Plus. Antimicrobial susceptibility testing (AST) of *Acinetobacter* spp. was performed by Kirby–Bauer disk diffusion method and MicroScan Walkaway 96 Plus ID/AST system. Colistin susceptibility was performed by MicroScan Walkaway 96 Plus system. Results of AST were interpreted as per Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines.

Results

Baseline Characteristics

Out of the 100 isolates, 63% of bacterial isolates were obtained from male patients with male to female ratio of 1.70:1 (►**Table 1**). The mean age of patients was 25 ± 20.99 years ($\pm$ standard deviation). Most common age group was 31 to 60 years (56%).

Out of the 100 isolates tested, 35% were from critical areas, 39% from medical wards, and 26 from surgical wards (►**Table 1**).

Maximum isolates were obtained from endotracheal (ET) aspirate (34%), followed by pus (26%), sputum (9%), BAL (9%), blood (8%), urine (6%), biopsy tissue (2%), and body fluids (6%); 59% presented with lower respiratory tract infections (LRTIs), 54% presented with pyrexia of unknown origin, 53%

Table 1 Baseline characteristics

Age (y)	1–14	15–30	31–60	>60		
N (%)	10 (10)	15 (15)	56 (56)	19 (19)		
Ward distribution	HDU	CCU	NICU	ICU	Medical wards	Surgical wards
N (%)	15 (15)	11 (11)	5 (5)	4 (4)	39 (39)	26 (26)
Clinical features	LRTI	PUO	UTI	BSI		
N (%)	59 (59)	54 (54)	53 (53)	34 (34)		

Abbreviations: BSI, blood stream infection; CCU, critical care unit; HDU, high dependency unit; ICU, intensive care unit; LRTI, lower respiratory tract infection; NICU, neonatal intensive care unit; PUO, pyrexia of unknown origin; UTI, urinary tract infection.
Note: Table of multiple response will exceed 100%.

presented with UTIs, and 34% presented with blood stream infections (►**Table 1**).

Agreement Analysis

Out of the 100 isolates, 92% were identified as *ABC* and 3% were identified as *Acinetobacter lwoffii* by both MicroScan WalkAway and MALDI-TOF.

There was 5/100 (5%) discrepancy in agreement between MicroScan WalkAway and MALDI-TOF and of these 5 isolates 2 were identified as *Stenotrophomonas maltophilia* by MicroScan WalkAway but as ABC by MALDI-TOF.

Three (3%) were identified as *ABC* by MicroScan WalkAway, while they were identified as non-ABC (*Acinetobacter junii*, *Acinetobacter johnsonii*, *Acinetobacter tandoii*) by MALDI-TOF.

Out of the 100 isolates, 94% were identified as *ABC* by both CBM and MALDI-TOF and 3% were identified as *A. lwoffii* by both CBM and MALDI-TOF. There was 3% discrepancy in agreement between CBM and MALDI-TOF, that is, they were identified as *ABC* by CBM, while they were identified as non-ABC (*A. junii*, *A. johnsonii*, *A. tandoii*) by MALDI-TOF.

Out of the 100 isolates, 95% were identified as *ABC* by both CBM and MicroScan WalkAway and 3% were identified under *A. lwoffii* by both CBM and MicroScan WalkAway. There was 2% discrepancy in agreement between CBM and MicroScan WalkAway which were identified as *ABC* by CBM, while they were identified as *S. maltophilia* by MicroScan WalkAway (►**Table 2**).

Coefficient of agreement (kappa) between CBM, MicroScan WalkAway, and MALDI-TOF was calculated and value interpreted (►**Table 3**).

Antibiotic Susceptibility Results

Out of the 100 isolates, 2% of *ABC* and 3% of non-*ABC were found to be* sensitive to all the drugs tested. One percent of *A. baumannii was found to be* multidrug resistant (MDR); 91% of *ABC* (90% *A. baumannii* and 1% *Acinetobacter nosocomialis*) were found to be extensive drug resistant (XDR); 3% of non-*ABC* (one each of *A. lwoffii*, *A. johnsonii*, and *A. tandoii*) were found to be XDR.

For all antibiotics, there was no significant difference in susceptibility detected by both Kirby–Bauer disk diffusion method and MicroScan WalkAway (►**Table 4**).

We found that there was a 100% categorical agreement between both systems with respect to susceptibility of aminoglycosides (amikacin, gentamicin, tobramycin) and cephalosporins (ceftazidime, cefepime, cefotaxime). Total of 16 errors were observed out of which 2 were very major errors (MEs), seen with ampicillin–sulbactam and tetracycline. Six were MEs, seen with piperacillin–tazobactam, levofloxacin, imipenem, meropenem, and cotrimoxazole. Eight were minor errors, found with piperacillin–tazobactam. levofloxacin, imipenem, meropenem, and tetracyclines.

For colistin, the Kirby–Bauer disk diffusion method was not done, MicroScan WalkAway 96 Plus system results were interpreted as per CLSI M100 guidelines. Out of 100 isolates, 99 (99%) were found sensitive and 1 (1%) was found intermediate to colistin.

Discussion

In recent few years, *Acinetobacter* species has emerged as an important pathogen along with an increasing trend toward drug resistance.

In our study, *Acinetobacter* infection was found to be more common in males, with male to female ratio of 1.70:1, which is similar to other studies and mainly belonged to 31 to 60 years (56%). A similar trend was noted in study conducted by Cucunawangsih et al where maximum isolates (73.8%) were obtained from age group of 40 to 60 years[13] and in contrast, Dimple and Nupur reported maximum isolation from the age group of <10 years (22.6%) followed by age group of 41 to 50 years (20.8%).[14] The trend seen in our study may be because patients in the age group >40 years have more chances of having comorbid conditions making them more prone to bacterial infections such as MDR *Acinetobacter* infections.

In the present study, most of the cases were from medical wards and specialized/critical care units. Similar findings were seen in study by Dimple and Nupur, Tripathi et al, and Rajmane et al reported higher isolation from intensive care units.[14–16]

Isolation of majority of *Acinetobacter* species was from LRTIs (59%), and majority of isolates were obtained from respiratory samples (51%) especially ET aspirates (34%). Several other studies show that *Acinetobacter* species were mainly obtained from respiratory samples.[17,18] Current

Table 2 Comparative results of various methods in identification of *Acinetobacter* species

Organism identified	*Acinetobacter* species	MALDI-TOF				MicroScan			Conventional BCM
		Total identified	Log score			Total identified	Percent probability		
		N	>2 *N* (%)	1.7–1.99 *N* (%)	<1.7 *N* (%)	*N*	High probability (> 85%) *N* (%)	Low probability (< 85%) *N* (%)	
ABC	*A. baumannii*	92	77 (83.69)	14 (15.2)	1 (1.08)	95 92 + 3[a]	95 (95)	–	**94**
	A. nosocomialis	2[b]	1 (50)	1 (50)	–	–	–	–	
Non-ABC	*A. lwoffii*	3	1 (33.33)	2 (66.66)	–	3 (3)	3 (3)	–	6
	A. junii	1[a]	1 (100)	–	–	–	–	–	
	A. johnsonii	1[a]	1 (100)	–	–	–	–	–	
	A. tandoii	1[a]	–	–	1 (100)	–	–	–	
	Stenotrophomonas maltophilia	–	–	–	–	2[b]	–	–	
Total		100 (100)	81 (81)	17 (17)	2 (2)		–		100

Abbreviations: ABC, *Acinetobacter baumannii* complex; BCM, biochemical methods; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight; NABC, non-*Acinetobacter baumannii* complex.
Notes: >2—high confidence identification. 1.7 to 1.99—low confidence identification. <1.7—nonidentification.
[a]Three isolates identified as ABC by MicroScan were identified as NABC.
[b]*Stenotrophomonas maltophilia* identified by MicroScan were identified as ABC by MALDI-TOF.

Table 3 Agreement analysis

Organism	*Acinetobacter baumannii* complex	Non-*Acinetobacter baumannii* complex	Agreement (%)	Kappa coefficient
Identified by both MicroScan WalkAway and MALDI-TOF, *N* (%)	92 (92%)	3 (3%)	95	0.519
Identified by both conventional biochemical tests and MALDI-TOF, *N* (%)	94 (94)	3 (3)	97	0.653
Identified by both conventional biochemical tests and MicroScan WalkAway, *N* (%)	95 (95)	3 (3)	98	0.740

Abbreviation: MALDI-TOF, matrix-assisted laser desorption ionization–time of flight.
Notes: Kappa values < 0—no agreement, 0.00 and 0.20—slight agreement, 0.21 and 0.40—fair agreement, 0.41 and 0.60—moderate agreement, 0.61 and 0.80—substantial agreement, and 0.81 and 1.00—almost perfect agreement. There was moderate agreement (kappa coefficient—0.519) between identification by MicroScan WalkAway and MALDI-TOF. There was substantial agreement (kappa coefficient—0.653) between conventional biochemical tests and MALDI-TOF. There was substantial agreement (kappa coefficient—0.740) between conventional biochemical tests and MicroScan WalkAway.

findings strengthens the evidence that *Acinetobacter* infections are one of the most common causes of hospital acquired respiratory tract infections, due to their propensity to survive and persist in moist environment such as suction tubes, ventilators for a long period of time and due to MDR to commonly used antibiotics.

Conventionally, isolates which were nonlactose fermenting, oxidase negative, nonmotile, gram-negative coccobacilli,

Table 4 Categorical agreement between by Kirby–Bauer disk diffusion and MicroScan WalkAway 96 Plus system

Antibiotics	MicroScan WalkAway 96 Plus system *n*/*N*	Kirby Bauer disk diffusion *n*/*N*	Categorical agreement (%)	VME (%)	ME (%)	mE (%)	Odds ratio	*p*-Value (95% confidence interval)
Aminoglycosides								
Amikacin	0/5	0/5	100	0	0	0	1	1 (0.28–3.56)
Gentamicin	0/6	0/6	100	0	0	0	1	1 (0.31–3.21)
Tobramycin	0/6	0/6	100	0	0	0	1	1 (0.31–3.21)
β-lactam/β-lactamase inhibitors combinations								
Ampicillin–sulbactam	1/6	0/6	83.33	16.67	0	0	1.18	0.77 (0.38–3.6)
Piperacillin–tazobactam	0/5	1/5	78.94	0	20	1.06	1	1 0.31–3.21)
Fluoroquinolones								
Ciprofloxacin	0/5	0/5	100	0	0	0	1	1 (0.28–3.56)
Levofloxacin	0/8	1/8	86.42	0	12.5	1.08	0.73	0.58 (0.25–2.19)
Cephalosporins								
Ceftazidime	0/7	0/7	100	0	0	0	1	1 (0.34–2.96)
Cefepime	0/6	0/6	100	0	0	0	1	1 (0.31–3.21)
Cefotaxime	0/5	0/5	100	0	0	0	1	1 (0.28–3.56)
Carbapenems								
Imipenem	0/6	1/6	81.16	0	16.67	2.17	0.73	0.58 (0.25–2.19)
Meropenem	0/6	1/6	80.04	0	16.67	3.29	0.82	0.75 (0.24–2.79)
Folate pathway inhibitors								
Cotrimoxazole	0/8	2/8	75	0	25	0	0.73	0.58 (0.25–2.19)
Tetracyclines								
Tetracycline	1/6	0/6	78.93	16.67	0	1.07	1	1 (0.34–2.96)

Abbreviations: ME, major error; mE, minor error; *n*, number of discrepant sensitive results; *N*, total number of sensitive isolates; VME, very major error.

and citrate utilizer are identified as *Acinetobacter* species. However, the emergence of resistant strains of *A. baumannii* raised the need for further speciation of the bacteria. The currently available CBMs can only broadly classify them into *A. calcoaceticus*–ABC and *A. lwoffii*. The phenotypic techniques which are currently available are insufficient in accurately identifying and differentiating the closely related and clinically important *Acinetobacter* species. Moreover, it is tedious and time consuming.

Although various molecular methods are available, they are not regularly used in diagnostic laboratories. Automated systems such as MicroScan WalkAway 96 Plus ID/AST system have been introduced to overcome these difficulties, but it has also failed to distinguish *Acinetobacter* species further. MALDI-TOF MS is a proteomic-based method, which provides rapid species identification of *Acinetobacter* strains. MALDI-TOF is increasingly used in diagnostic microbiology for the routine identification of bacteria till the species level.[8]

In our study, we performed a comparative evaluation of CBMs, MALDI-TOF MS and MicroScan WalkAway 96 Plus automated systems for the correct identification of *Acinetobacter* species. We although found that there was a substantial agreement between CBMs with MALDI-TOF MS (kappa 0.653, $p < 0.05$) and MicroScan WalkAway 96 Plus automated systems (kappa 0.74, $p < 0.05$). Majority of *A. baumannii* identified with biochemical methods were concordant with MALDI-TOF and MicroScan WalkAway systems currently, while other *Acinetobacter* species had discordant results.

We found a moderate agreement between MALDI-TOF MS and MicroScan WalkAway 96 Plus ID/AST system (kappa 0.519, $p < 0.05$) for identification of *Acinetobacter* species. This drop in agreement is due to discordant results between the two systems in identification of not only other *Acinetobacter* species but also *A. baumannii* few of which were identified as *S. maltophilia* in Microscan Walkaway system. Study by Lee et al suggested that the MALDI-TOF is more advantageous than MicroScan WalkAway 96 Plus ID/AST system and VITEK 2 for routine diagnosis in clinical microbiology laboratories. Furthermore, an isolate not primarily identified by MALDI-TOF can be retested by using other automated methods. In addition, the low rate of misidentification obtained by MALDI-TOF is an advantage along with offering more rapid and reliable ID of other bacterial isolates.[19]

In our study, MALDI-TOF was able to speciate the organism as *A. baumannii*, *A. nosocomialis*, *A. lwoffii*, *A. junii*, *A. johnsonii*, and *A. tandoii*; 81 (81%) of the isolates were identified with high confidence level, 17 (17%) of the isolates were identified with low confidence level, and 2 (2%) were not identified. For these two isolates, the best match among the choices provided was taken as the ID, that is, *A. baumannii* and *A. tandoii*, which was similar to the studies by Hsueh et al, Espinal et al, and Šedo et al.[20–22] Hence, we too infer that MALDI-TOF has an inherent limitation in identifying genus *Acinetobacter* up to species.

MicroScan WalkAway 96 Plus ID/AST system can identify *Acinetobacter* species as *ABC* and *A. lwoffii* group but cannot speciate it further. In our study, MicroScan WalkAway 96 Plus ID/AST system identified 95% isolates as ABC, 3% isolates as *A. lwoffii* group, and 2 (2%) isolates as *S. maltophilia* with low probability ID. Low probability indicates that the chances of the isolate being *S. maltophilia* is low and it may be an erroneous result. There is a paucity of published studies on performance of MicroScan WalkAway 96 Plus ID/AST system. One study by Hernandez-Duran et al showed that there was 89% concordance of identification by MicroScan WalkAway system with CBMs and VITEK 2 system, as compared with VITEK 2, MicroScan system presented a longer delay in obtaining results and greater difficulty in the correct identification of gram-negative bacteria including *Acinetobacter* species.[23]

Common isolates in the present study were *A. baumannii* (92%) and *A. nosocomialis* (2%) belonging to *ABC* (94%). Other species isolated were *A. lwoffii* (3%), *A. junii* (1%), *A. johnsonii* (1%), and *A. tandoii* (1%). Three *A. lwoffii* and one each of *A. junii*, *A. johnsonii* were obtained from ET aspirate and pus, respectively. Few studies have reported these agents where confirmation has been obtained by gene sequencing.[6,24] A single *A. tandoii* was isolated from a urine sample in our study with low confidence, hence further molecular studies are necessary for the identification of species. Karah et al isolated *A. tandoii* from blood sample by rpoB sequencing, but it was later found to be *Acinetobacter baylyi* after 16S rDNA gene sequencing suggesting that *A. tandoii* may not be a pathogen.[25]

In our study, we compared the antibiotic sensitivity results of Kirby–Bauer disk diffusion method with MicroScan WalkAway 96 Plus ID/AST system. When there was discrepancy in two methods, Kirby–Bauer disk diffusion method's results were taken as reference standard. We found that there was a 100% categorical agreement between both systems with respect to susceptibility of aminoglycosides and cephalosporins. Total of 16 errors were observed. These results were similar to other studies where a substantial number of very major and major errors were also reported.[18,26,27] Habib Babay et al suggested the due to different carbapenem resistance mechanisms among *Acinetobacter* species may lead to disparity in detection of resistance by Kirby–Bauer and MicroScan WalkAway 96 Plus system. Hence, a second, independent antimicrobial susceptibility testing method to validate susceptibility results may be used where possible, especially in a setting of critically ill patients with bacterial infection when only automated systems are used.[5]

MDR *Acinetobacter* species are defined as resistance to more than two of the following five drug classes: antipseudomonal cephalosporins (ceftazidime or cefepime), antipseudomonal carbapenems (imipenem or meropenem), ampicillin–sulbactam, fluoroquinolones (ciprofloxacin or levofloxacin), and aminoglycosides (gentamicin, tobramycin, or amikacin). XDR *Acinetobacter* species are defined as MDR *Acinetobacter* species which show additional resistance to carbapenems. Pan drug-resistant *Acinetobacter* species are defined as XDR *Acinetobacter* species that are also resistant to polymyxins and tigecycline.[28] In the present study, 94% of *Acinetobacter* species were found to be XDR, 5% were found to

be sensitive to all the drugs tested, and one was found to be MDR. This is similar to other studies; however, occasional reports have shown majority of *Acinetobacter* species to be sensitive to meropenem and amikacin. This difference may be attributed to the variations in geographical distribution of various *Acinetobacter* species, their sensitivity patterns, and laboratory quality control practices.[15,17,18,29]

Increasing use of colistin for MDR gram-negative bacterial infections has led to the emergence of colistin resistance in several countries worldwide which may vary between regions, with majority studies showing resistance rates of $< 10\%$.[30] In our study, MicroScan WalkAway 96 Plus system based on broth microdilution (BMD) method was used and the results were interpreted as per CLSI 2019 guidelines.[31] Out of 100 isolates,99% were found sensitive to colistin and only 1% was found intermediate. Since the joint CLSI and EUCAST Polymyxin Breakpoints Working Group recommend only reference BMD method as the reference test for determining susceptibility to colistin the Kirby–Bauer disk diffusion method was not done.[32]

Jayol et al stated that there is a high rate of major errors (26.9%) observed with the MicroScan WalkAway 96 Plus ID/AST system when compared with manual BMD, due to an overestimation of the minimum inhibitory concentrations (MICs) for the nonfermenting gram-negative bacilli including *Acinetobacter* species.[33] In a study conducted in our institute by Singh et al, categorical agreement between MicroScan WalkAway 96 Plus ID/AST system and Mikrolatest MIC AST kit was found to be 71.4% for *A. baumannii* and 100% for *A. junii* and *A. johnsonii*. Total two major errors for *A. baumannii* were observed.[34] This shows that even though automated systems are less tedious, it has its own inherent errors. The automated systems depend on the library of information for their results, and if the organism provided matches another organism in the system, the results will be inconsistent, which has to be confirmed with further testing. Hence, even after advent of various automated systems, the role of microbiologists will not decrease as they are required to interpret the results correctly and as applicable. Furthermore, routine infection control practices have a role in control of MDR and XDR infections as the hospital equipment could serve as a reservoir for *Acinetobacter* species.

The limitations of the current study were as follows: The gold standard of identification of *Acinetobacter* species is gene sequencing and the reference test for determining susceptibility in the form of MIC to colistin is BMD. However, these could not be performed due to logistical issues.

While MALDI-TOF identification costs few rupees, other automated systems cost in hundreds and molecular methods cost thousands, which in a developing country like India amount to a huge financial burden to the patients.

Conclusion

In conclusion for the species identification of *Acinetobacter* strains, MALDI-TOF is a rapid, reliable method and can thus be routinely used in clinical laboratories.

Phenotypic methods are currently used to determine the antibiotic susceptibility of bacterial isolates. Various commercial automated systems are widely used routinely in clinical laboratories. Although these systems are rapid, convenient, and decrease manpower requirement, they have inherent limitations. Hence, standardized techniques and quality control practices have to be followed in these settings.

While it is not prudent to completely automate, we can use these methods along with conventional phenotypic methods of identification and AST. The feedback from the conventional phenotypic methods can be used to further upgrade the automated systems.

Authors' Contribution

P.G., A.K., M.B., and B.J.O. conceptualized and designed the study; and definition of intellectual content, literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review were also done by them. P.G. was the guarantor.

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Conflict of Interest

None declared.

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