ORIGINAL ARTICLE

PERFORMANCE OF THE VITEK MS MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY SYSTEM FOR RAPID IDENTIFICATION OF CLINICAL MICROBIOLOGY ISOLATES

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ABSTRACT

Background: Rapid and precise microbial identification is essential for timely patient management and control of infection. Conventional microbiological procedures are time-consuming, laborious, and require expertise. Recent coalition of MALDI-TOF in the microbiology workflow has yielded excellent results so far. Here we have assessed this quick proteomic based technique for clinical bacterial isolates identification.

Material and Methods: All fresh bacterial isolates were selected for VITEK MS (bioMérieux, France*)* analysis, the bacteria were identified by using MALDI-TOF System (IVD 3.2) software. For same isolates, API (bioMérieux, France*)* was set as per kit instructions and conventional/biochemical tests were performed according to ASM Guidelines.

Results: Among 200 isolates 99% were accurately identified by the VITEK MS system to the species level, one of two isolates was mis-identified (*Shigella* as *E. coli*) while the other was identified later by re-spotting. On testing these samples in parallel by APIs, 91.50% were correctly identified, while 8.50% (17 samples) showed discrepant results. These were re-analyzed by VITEK-2 (bioMérieux, France*)* semi-automated system which showed the same results as those of VITEK MS. Our findings revealed diagnostic accuracy of VITEK MS in comparison with APIs in terms of time, cost and patient management.

Conclusion: For bacterial identification MALDI-TOF MS is an expeditious, authentic and comparatively inexpensive system. Our results emphasize that it is speedy technique which can replace the traditional identification methods for most of bacterial strains on their routine isolation. This ingenious approach complies with advanced patient management and therapeutic intervention.

Key Words: MALDI-TOF MS, bacterial identification, VITEK-2, Analytical profile index (API), Clinical microbial isolates

BACKGROUND

Due to substantial increase in drug resistance, prompt bacterial identification is imperative for the initiation of antimicrobial treatment and infection prevention.[1] Conventional identification methods are laborious, and have a long turnaround time as these involve observational procedures like growth on appropriate medias, colony appearance, Gram stain results, microscopy, and biochemical reactions. However molecular methods are not commendable for extensive routine identification. Accurate and quick microbial identification provides exact knowledge about the infectious cause. Un-identified organisms could also be identified using DNA sequencing by reference laboratories. Currently semi-automated biochemical test programs like VITEK-2 (bioMérieux, France*)* are commonly used in analytical microbiology laboratories alongside regular culture methods for day-to-day microbial isolation thus decreasing mean processing time up to a few hours. Although, cost of the reagents is a limiting factor in the laboratory setting of a developing country, like Pakistan.[2,3]

Genomics and proteomics are among various methods used to study bacterial macromolecules (genome, protein, polysaccharides, phospholipids). A genomic method gives us extensive information about bacterial genome while proteomics helps us to recognize multiple bacterial proteins.[4] VITEK MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry uses proteomics, to study bacterial protein content and provides detailed information about the cell and helps in identifying microorganism including mycobacterium, viruses,

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parasites, molds and yeast. This instrument along with antimicrobial susceptibility testing results could prove helpful in early patient management, monitoring and epidemiological surveillance.[5]

MALDI-TOF MS instrument was officially launched for diagnostic use in clinical microbiology department in Chughtai Laboratory, Lahore in January 2021. The aim of our study is to assess the performance and diagnostic accuracy of the VITEK MS (bioMérieux, France) on clinical microbiology aerobic and anaerobic isolates by processing 200 samples during a 6-months period (February -July 2022).

MATERIAL AND METHODS

After approval from institutional review board (Letter No CIP/IRB/1101) this cross-sectional study was conducted over a six-month period February 2022 to July 2022 at the Chughtai Laboratory Lahore, Pakistan. Approximately 200 samples were processed using non-probability convenience sampling technique (aerobic/anaerobic), clinical isolates were recovered from different clinical samples including blood, urine, CSF, wound, and sputum.

Before subjecting isolates to MALDI-TOF MS, they were initially isolated on selective/non-selective media as 5% Blood agar plate (BAP), Chocolate agar (CHOC), and MacConkey agar (MAC) for aerobes and Sheep blood agar (SBA) for anaerobes. Respective plates were incubated aerobically for 18-24 hours and 48-72 hours for anaerobes under 35C incubation shown in Figure-I.

All isolates were identified with conventional/biochemical tests as per ASM guidelines. The APIs or Analytic Profile Index classifies bacteria on the basis of biochemical reactions, allowing rapid identification of known bacteria (Figure-II). Results of APIs were compared with VITEK MS. Any discrepancies when occurred were subsequently resolved by semi-automated biochemical test platform, VITEK-2.

Clinical isolates identification done by VITEK-MS system utilizing direct single deposit from bacterial colonies in absence of prior extraction according to manufacturer's guidelines as shown in Figure-III.

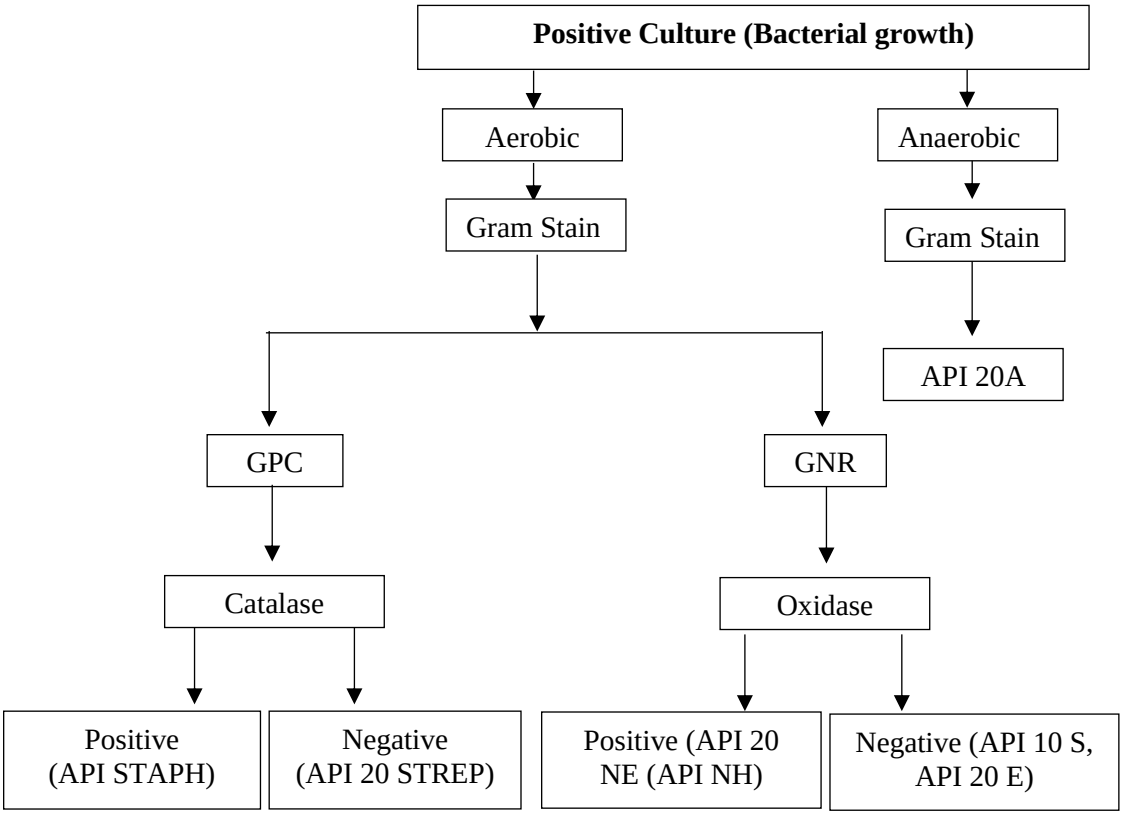

Figure -I: Workflow chart for conventional identification of isolates.

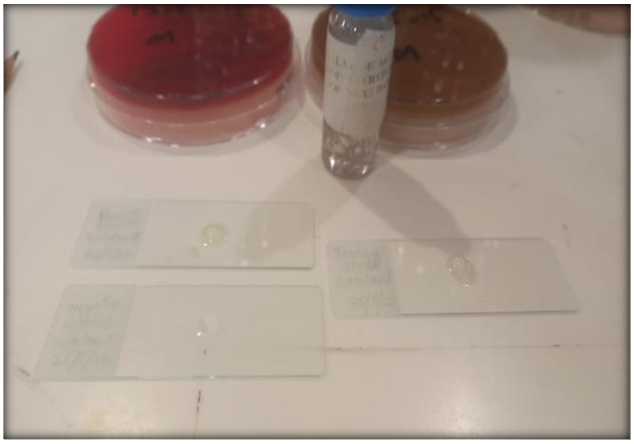

Figure-II: API System for identification of clinical bacterial isolates.

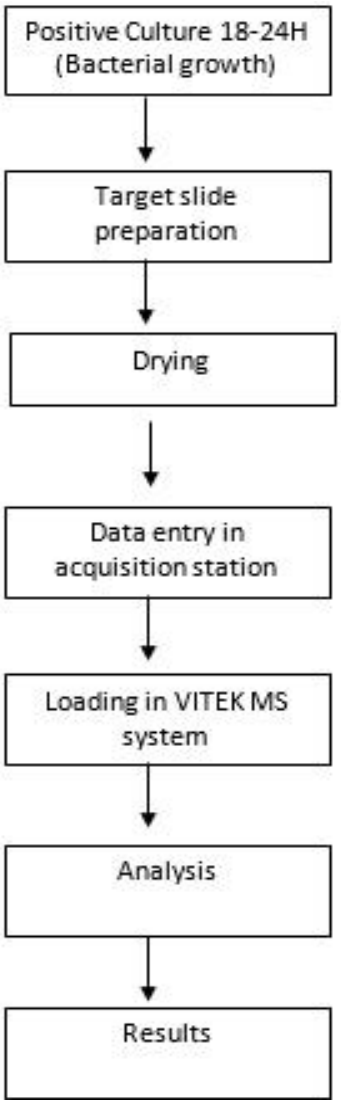

Figure-III: VITEK MS (bioMérieux, France) system.

RESULTS

200 isolates were analyzed by the VITEK MS (IVD 3.2, >15000 bacterial spp. in database) during six months study period and in parallel by the conventional/biochemical tests, APIs. Among all the bacterial isolates (*Enterobacterales*, Non-fermentor, Gram-negative rods, *Staphylococci*, *Streptococci*,

Enterococcus and other GPCs, Anaerobes) 198/200 (99%) were correctly identified by the VITEK MS system with confidence value 99.9% as shown in Table-I. On account of remaining two isolates, one was misidentified (*Shigella* as *E.coli*) while another was unidentified first due to insufficient quantity of colony while spot preparation however identified later by re-spotting which emphasizes the fact that improper spot can result in poor identification.

APIs with regular biochemical tests were also performed simultaneously on the same 200 isolates along with controls which showed correct identification for 183/200 (91.50%) isolates. While analyzing the results of VITEK MS and conventional system, 17 samples (8.50%) showed discrepant result. The discrepant results were analyzed by VITEK-2 Semi automation and the obtained results found in agreement with VITEK MS results as shown in Table-II. Semi-automated VITEK 2 system was used to cope discrepancies and to provide correct identification up to specie/subspecies level. Comparison of APIs and VITEK MS identification is shown in Figure-IV.

Table-I: Identification comparison between VITEK MS and API systems.

Clinical bacterial isolate	Number of isolates	Correct identification		Misidentification		No identification	
		VITEK MS	API	VITEK MS	API	VITEK MS	API
		Enterobacterales					
Citrobacter amalonaticus	1	1	1				
Citrobacter freundii	1	1	1				
Enterobacter aerogenes	1	1	1				
Enterobacter cloacae	6	6	6				
Enterobacter fergusoni	1	1					1
Enterobacter hormaechei	3	3					3
Escherichia coli	36	36	36				
Klebsiella pneumoniae	18	18	18				
Morganella morganii	5	5	5				
Pantoea dispersa	1	1					1
Proteus mirabilis	2	2	2				
Proteus vulgaris	1		1			1	
Providencia rettgeri	2	2	2				
Providencia stuarti	1	1	1				
*Salmonella enterica**	11	11	11				
Serratia marcescens	19	19	19				
Shigella sonnei	1		1	1			
Non-fermenters							
Acinetobacter baumannii	13	13	13				
Acinetobacter calcoaceticus	1	1	1				
Acinetobacter haemolyticus	1	1	1				
Acinetobacter junii	1	1	1				
Acinetobacter pitti	2	2					2
Achromobacter denitrificans	1	1					1
Aeromonas jandaei	1	1					1
Aeromonas punctata	1	1					1
Aeromonas sobria	1	1	1				
Burkholderia cenocepacia	1	1					1
Burkholderia cepacia	1	1	1				
Burkholderia contaminans	6	6					6
Chryseobacterium indologenes	1	1	1				
Pseudomonas aeruginosa	8	8	8				
Pseudomonas mendocina	2	2	2				
Pseudomonas putida	1	1	1				
Pseudomonas stutzeri	4	4	4				
Rhizobium radiobacter	1	1	1				
Stenotrophomonas maltophilia	13	13	13				
		Staphylococci					
Staphylococcus aureus	8	8	8				

Staphylococcus capitis	1	1	1
Staphylococcus cohnii	1	1	1
Staphylococcus haemolyticus	3	3	3
Staphylococcus hominis	1	1	1
Staphylococcus saprophyticus	1	1	1
Staphylococcus sciuri	1	1	1
Streptococci			
Streptococcus agalactiae	2	2	2
Streptococcus anginosus	1	1	1
Streptococcus dysgalactiae	1	1	1
Streptococcus mitis	2	2	2
Streptococcus pneumoniae	2	2	2
Streptococcus pyogenes	1	1	1
Enterococcus* & other *GPC			
Enterococcus faecalis	1	1	1
Micrococcus luteus	2	2	2
Anaerobe			
Bacteroides thetaiotaomicron	1	1	1
HACEK			
H. influenzae	1	1	1

Table-II: Conformation of discrepant results by VITEK-2 semi-automated system.

API result	VITEK-MS result	VITEK-2 result	Total (n=17)
Enterobacter spp.	*Enterobacter fergusoni*	*Enterobacter fergusoni*	1
Enterobacter spp.	*Enterobacter hormaechei*	*Enterobacter cloacae complex**	3
Pantoea spp.	*Pantoea dispersa*	*Pantoea spp.**	1
Acinetobacter spp.	*Acinetobacter pitti*	*Acinetobacter baumannii complex**	2
Achromobacter spp.	*Achromobacter denitrificans*	*Achromobacter denitrificans*	1
Aeromonas spp.	*Aeromonas jandaei*	*Aeromonas jandaei*	1
Aeromonas spp.	*Aeromonas punctata*	*Aeromonas punctata*	1
Burkholderia spp.	*Burkholderia cenocepacia*	*Burkholderia cenocepacia*	1
Burkholderia spp.	*Burkholderia contaminans*	*Burkholderia contaminans*	6
Shigella sonnei	*Escherichia coli*	*Shigella sonnei*	1

Table-III: Time dynamics for VITEK-MS.

Workflow	Time (seconds)
Slide spot preparation	20
Drying	120
Matrix application	10
Drying	120
Loading into system	60
Result time	40
Total time	**370 (6minutes,10seconds)**

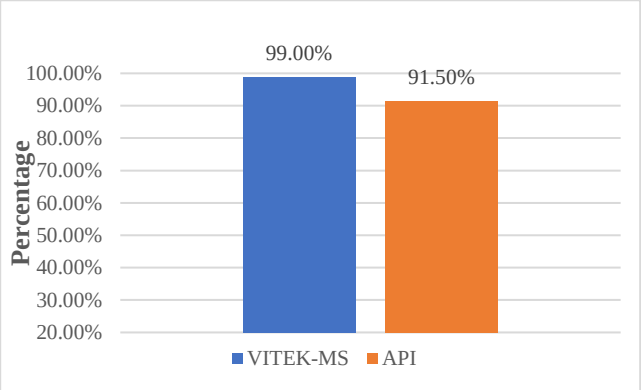

Figure-IV: Comparison of API and VITEK-MS identification percentage.

DISCUSSION

MALDI-TOF MS, is a rapid and sensitive technique for identifying clinical microbiological isolates. This study proposes bacterial identification comparison between VITEK MS and APIs, performed at Chughtai Laboratory Lahore, Pakistan. The VITEK MS (bioMérieux, France) is an advance automated system, without need for intensive background training for its operator.

In our study on comparing the identification of clinical samples, VITEK MS correctly identified 99%, while APIs up to 91.50%, whereas 8.50% results were

discordant. Some of the bacterial species such as *Citrobacter amalonaticus, Acinetobacter junni, Rhizobacterium radiobacter, Acinetobacter calcoaceticus* and *Micrococcus*, identified by APIs, were only possible with the help of certain additional tests. The results of APIs were the same as that of MALDI-TOF in terms of genus identification. However, APIs could not speciate the isolates to the level of accuracy of MALDI-TOF. Although accuracy of VITEK MS was 99%, but in case of certain bacteria, such as *Salmonella spp*. further serological testing is warranted. Literature review has revealed that MALDI-TOF is a safe and accurate method of bacterial identification with markedly reduced biowaste production. In comparison to the current study previous studies have documented non-clinical isolates .[6-10,12-14] Furthermore, additional benefit of using MALDI-TOF is that it's a safe option to identify hazardous pathogens (*Brucella spp, Bacillus anthracis, Francisella tularensis*) due to shorter handling time, absence of aerosol generating steps.[15,16]

In present study primary focus was centered on clinical bacterial isolates identification. In comparison with MALDI-TOF, API is a conventional method but it has the advantage of identifying *salmonella enterica subspecies enterica* serotypes without need for an additional serogrouping, however it requires 24-48 hours for analysis, and late identification results in delay of both empiric and targeted treatment. This means that rapid, reliable, cost-effective procedures are important prerequisites of a clinical microbiology.[11] In our study VITEK MS has analyzed the samples within few minutes (6min 10 seconds). The time dynamics for MALDI-TOF were calculated and shown in the Table-III. In addition to this reduced running cost, technical expertise, provision of the authentic results gives an edge to the VITEK MS over other identification procedures. However, there are certain precautionary measures which include careful application, avoiding spillage of matrix between spots and avoiding marking on slide.[6,11]

There were a few limitations of this study. Firstly, the current study was single centered, further validation with a similar study containing large number of samples(multicentric) should be done. Secondly, discrepant results could have been more precisely confirmed by 16s rRNA sequencing.

CONCLUSION

Addition of the VITEK MS to our laboratory facilitates turnaround time and adds in diagnostic efficiency for bacterial identification. It was observed that rapid bacterial identification can affect patient's management. Number of database entries are crucially important for reliable identification of microorganisms thus continuous update of database software is mandatory for excellent performance. MALDI-TOF is a game changer. It seems to play a vital role in future clinical microbiological laboratories in Pakistan. More research is required to achieve efficient identification directly from samples like blood, CSF, Pus and urine cultures along with antibiotic susceptibilities.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

CONTRIBUTION

Alina Mehwish: Original concept and design of work; the acquisition, drafting, revision.

Irim Iftikhar: Critical revision, final approval of version, questions related to accuracy/integrity of different parts of work resolution.

Karam Rasool: Analysis, interpretation of data for the work.

REFERENCES

1. Dubois D, Grare M, Prere MF, Segonds C, Marty N, Oswald E. Performances of the Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. J Clin Microbiol. 2012; 50(8): 2568-76. DOI: 10.1128/JCM.00343-12
2. Luo Y, Siu GK, Yeung AS, Chen JH, Ho PL, Leung KW, *et al*. Performance of the VITEK MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid bacterial identification in two diagnostic centres in China. J Med Microbiol. 2015; (64): 18-24. DOI: 10.1099/jmm.0.080317-0
3. Mitchell SL, Alby K. Performance of microbial identification by MALDI-TOF MS and susceptibility testing by VITEK 2 from positive blood cultures after minimal incubation on solid media. Eur J Clin Microbiol Infect Dis. 2017; 36 (11): 2201-6. DOI: 10.1007/s10096-017-3046-0
4. Alizadeh M, Yousefi L, Pakdel F, Ghotaslou R, Rezaee M. A, Khodadadi E, Oskouei M. A., Soroush Barhaghi M. H., & Kafil H. S. MALDI-TOF Mass Spectroscopy Applications in Clinical Microbiology. Advances in pharmacological and pharmaceutical sciences, 2021, 9928238. DOI://doi.org/10.1155/2021/9928238
5. Bilecen K, Yaman G, Ciftci U, Laleli YR. 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 Oct; :516410. DOI: 10.1155/2015/5164102015

1. Panda A, Ghosh AK, Mirdha BR, Xess I, Paul S, Samantaray JC, *et al*. MALDI-TOF mass spectrometry for rapid identification of clinical fungal isolates based on ribosomal protein biomarkers. J Microbiol Methods. 2015; 109: 93-105. DOI: 10.1016/j.mimet.2014.12.014
2. Bizzini A, Greub G. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clinical Microbiology and infection. 2010; 16(11): 1614-9.
3. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, *et al*. Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol. 2008;74(17):5402–7. DOI: 10.1128/AEM.02689-07
4. Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Böttger EC, Hombach M. Identification of gram-positive cocci by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry: Comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. J Clin Microbiol. 2013;51(6):1834–40. DOI: 10.1128/JCM.02654-12.
5. Shitikov E, Ilina E, Chernousova L, Borovskaya A, Rukin I, Afanas'ev M, *et al*. Mass spectrometry-based methods for the discrimination and typing of Mycobacteria. Infect, Genet Evol. 2012; 12(4): 838–45. DOI: 10.1016/j.meegid.2011.12.013
6. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-Tof Mass Spectrometry: An emerging technology for microbial identification and diagnosis. Front Microbiol. 2015;6. DOI: doi.org/10.3389/fmicb.2015.00791
7. Saleeb PG, Drake SK, Murray PR, Zelazny AM. Identification of mycobacteria in solid-culture media by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol. 2011; 49(5): 1790–4. DOI: 10.1128/JCM.02135-10
8. Lotz Aurélie, Ferroni Agnès, Beretti J-L, Dauphin B, Carbonnelle E, Guet-Revillet Hélène, *et al*. Rapid identification of mycobacterial whole cells in solid and liquid culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2010;48(12):4481–6. DOI: 10.1128/JCM.01397-10
9. Fedorko DP, Drake SK, Stock F, Murray PR. Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis. 2012;31(9):2257–62. DOI: 10.1007/S10096-012-1563-4
10. Vranakis I, Papadioti A, Tselentis Y, Psaroulaki A, Tsiotis G. The contribution of proteomics towards deciphering the enigma of coxiella burnetii. Proteomics Clin Appl. 2013;7(1-2):193–204. DOI: 10.1002/prca.201200096
11. Lista F, Reubsaet FAG, De Santis R, Parchen RR, de Jong AL, Kieboom J, *et al*. Reliable identification at the species level of Brucella isolates with MALDI-TOF-MS. BMC Microbiol. 2011;11(1). DOI: doi.org/10.1186/1471-2180-11-267