Validation of MALDI-TOF Mass Spectometry for the Blood Stream Infection Focus on Outcomes Trial.

eP-768
23rd ECCMID
Apr 27th – Apr 30th, 2013
Berlin, Germany

J. Richards, M. Wootton, R. A. Howe, A. P. MacGowan

Introduction

Around 90-100,000 Blood Stream Infections (BSI) occur per annum in the UK at a cost of £6,200 per episode, with mortality up to 40%. Lack of BSI research leads to deficiency of information on outcomes/ risk factors for poor outcome. Patient factors related to underlying disease, site and severity of infection impact adversely on outcome whilst timely appropriate antimicrobial chemotherapy and removal of infected prosthetic materials are beneficial. Rapid laboratory based Diagnosis (RD) has beneficial effects on antibiotic use in infected patients and may reduce mortality. This multicentre trial aims to develop a programme of linked research studies aimed at improving management of BSI, reducing patient mortality. The RD technology chosen is Matrix Assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS). Validation of the MALDI-TOF Mass Spectrometry (MS) was required for bacterial identification from culture plates and direct from positive blood cultures

Table 1. MS results direct from cultures (no. with correct IDs/total).

Organism	Controls	Clinical	Scores
	No with correct ID / total		
S. aureus	7/7	153/153	2.2 – 2.4
CNS		43/43	2.2 – 2.4
E. coli	8/8	150/150	2.2 – 2.5
E. faecium	1/1	30/30	2.2 – 2.5
E. faecalis	1/1	37/37	2.2 – 2.5
S. pneumoniae	1/1	10/10	2.2 – 2.4
AHS		15/24	2.2 – 2.4
E. cloacae		82/82	2.1 – 2.4
E. aerogenes		9/9	2.2 – 2.4
E. asburiae		5/5	2.0 – 2.4
C. freundii		40/40	2.1 – 2.5
*C. koseri**		12/12	2.3 – 2.5
*K. pneumonia**	3/3	56/56	2.4 – 2.5
K. oxytoca		11/11	2.3 – 2.5
P. aeruginosa	3/3	23/23	2.2 – 2.5
P. putida	1/1	6/6	2.2 – 2.5
P. mirabilis	1/1	30/30	2.5 – 2.6
P. vulgaris		4/4	2.3 – 2.5
*Serratia sp**		38/38	2.1 – 2.5
A. baumannii		15/15	2.3 – 2.5
A. junnii		2/2	2.2 – 2.4
A. johnsonii		1/1	2.2
A. lwoffii		6/11	2.2 – 2.4
Candida sp	1/1	35/35	2.0 – 2.3

*** Incorrect on first occasion**

Citrobacter koseri

MALDI ID	Confidence	Consistency	Score
M. morganii	(+++)	A	2.576

10 repeats: C. koseri

Klebsiella pneumoniae

MALDI ID	Confidence	Consistency	Score
E. coli	(+++)	A	2.530

10 repeats: K. pneumoniae

Serratia marcescens

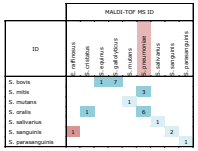
MALDI ID	Confidence	Consistency	Score
S. marcescens	(++)	B	2.192

10 repeats: S. marcescens

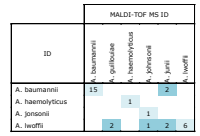
Rank (Quality)	Matched Pattern	Score Value
1 (++)	Morganella morganii	2.192
2 (++)	Morganella morganii	2.190
3 (++)	Morganella morganii	2.158
4 (++)	Serratia marcescens	2.148
5 (++)	Serratia marcescens	2.114
6 (++)	Serratia marcescens	2.092
7 (++)	Morganella morganii	2.035
8 (++)	Serratia marcescens	2.030
9 (+)	Serratia marcescens	1.959
10 (+)	Morganella morganii	1.943

Anomalies

Alpha haemolytic Streptococci



Acinetobacter species



Methods

854 previously identified BSI isolates including *S. aureus*, Enterobacteriaceae, Streptococci, & Candida were identified using MS with manufacturer's extraction method. 333 positive blood cultures (316 single organism (SO), 17 polymicrobial cultures (PM)) were processed using 3 programmes (Standard (SP), Blood Culture (BCP) & Blood Culture minus mixed culture feature (BCnomixP)) with manufacturer's Sepsityper kit, alongside conventional bacteriology. Any discrepancies were confirmed using 16S.

Table 2: Accuracy of MALDI-TOF MS for single organisms blood culture analysis.

TOTAL NON-MIXED samples	No of samples exhibiting confirmed result by STANDARD analysis	No of samples exhibiting confirmed results by BLOOD CULTURE analysis	No of samples exhibiting confirmed result by new BLOOD CULTURE (NO MIX) analysis
316	295 (93.4%)	291 (92.1%)	309 (97.8%)

Table 3: Single organism blood culture inaccuracies.

NON-MIXED CULTURES			
Non Ids	STANDARD analysis	BLOOD CULTURE analysis	BLOOD CULTURE (NO MIX) analysis
No ID	21 (6.7%)	5 (1.6%)	5 (1.6%)
False mixes	0	18 (5.7%)	0
No peaks	1 (0.3%)	2 (0.6%)	2 (0.6%)

Table 4: No reliable ID results with BCP

No reliable Ids				
Organism	Con	score	Target org in list	Position in list
CNS	C	1.476	Yes	4th
Bacteroides sp	C	1.142	No	
	C	1.504	Yes	2nd
	C	1.434	No	
Actinobaculum	C	1.842	Yes	1st

Results

Of 854 isolates from culture plates, MS accurately identified 99.9% to genus level, with 99.5% accurately identified on the first occasion. 9 out of 24 Alpha Haemolytic Streptococci (AHS) mis-identified as *S. pneumoniae*. Variations in Acinetobacter species were noted but were not considered significant (Table 1).

Of the 316 SO blood cultures 93.4% were accurately identified using the SP, with the majority of failures exhibiting "no reliable ID" (Table 4). The BCP and BCnomixP gave accurate IDs in 92.1% and 97.8% respectively (Table 2). The majority of inaccuracies using the BCP were due to false mixes (Tables 3 & 5). 35.3% of PM cultures were accurately identified using BCP. In all other PM cultures one of the species was accurately identified (Table 6).

Table 5: False mixed cultures / True mixed cultures with BCP

FALSE MIXED CULTURES			
Organism	Con	score	Other org in mix
S. aureus (3)	C	2.264	Lactobacillus sp
	A	2.363	Lactobacillus sp
	A	2.03	Arthrobacter sp
S. epidermidis (7)	A	2.26	Other CNS Staphylococcus
	A	2.341	Paenibacillus sp
	A	2.191	Bacillus sp
	A	2.138	Lactobacillus sp
	C	2.072	Paenibacillus sp
	A	1.818	Pseudomonas sp
	A	2.196	Other CNS Staphylococcus
S. haemolyticus (1)	A	2.337	Lactobacillus sp
Pasteurella sp (1)	A	2.233	Lactobacillus sp
Mycobacterium fortutitum (2)	A	2.149	Streptomyces sp
	A	2.042	Other mycobacterium
AHS (2)	B	1.794	Other Streptococcus
	B	2.361	Other Streptococcus
BHS) (1)	A	2.609	Lactobacillus sp
E. faecalis (1)	A	2.469	Paenibacillus sp

Table 6: True mixed polymicrobial cultures

TRUE POLYMICROBIAL CULTURES			
Culture	MALDI	Con	Score
Citrobacter koseri, Enterococcus faecalis, Escherchia coli, Group G Streptococcus	SINGLE	C	2.314
Clostridium perfringens + Klebsiella pneumoniae	MIX	A	2.308
Escherchia coli + Staphylococcus aureus	SINGLE	B	2.431
Proteus mirabilis + Morganella morgamii	MIX	A	2.57
Escherchia coli, Staphylococcus aureus, Coryneform species	SINGLE	B	2.25
Escherchia coli + Coagulase negative Staphylococcus	SINGLE	A	2.446
Aeromonas caviae + Escherchia coli	SINGLE	B	2.222
Veillonella atypica + Staphylococcus hominis	MIX	C	2.291
Enterobacter kobiae + Acinetobacter genomospecies	MIX	C	2.533
Enterococcus faecium, Staphylococcus haemolyticus + Pseudomonas aeruginosa	MIX	C	2.43
Staphylococcus warneri + Staphylococcus capitis	MIX	C	2.182
Escherchia coli + Actinobaculum schaalii	SINGLE	B	1.79

Conclusions

MS performed well for identification of bacterial BSI isolates from culture plates. AHS and anaerobes were problematic. For direct identity from blood cultures the MS performed better using the blood culture programme minus the mixed culture feature.

Specialist Antimicrobial Chemotherapy Unit, Public Health Wales, Cardiff, UK.
Email: mandy.wootton@wales.nhs.uk