Evaluation of the comparative performance of Verigene Blood culture Nucleic acid system to conventional techniques in a tertiary-care hospital in Kuwait.

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INTRODUCTION

- The diagnosis of bacteremia and sepsis is a priority in a Clinical Microbiology Department as they carry high mortality (20-50%). Early correct antibiotic treatment is correlated with higher survival rates. This is the main reason why broad spectrum antibiotics are usually administered until the microbiology results are known.
- Once the bacterial pathogen is known, treatment can be adjusted to a more specific antibiotic therapy.
- A key predictor of mortality rates in patients with severe blood stream infection is the time to identification of the causative pathogen and initiation of targeted therapy.
- Rapid identification of blood isolates is important in patient management as well as antimicrobial stewardship.
- The Verigene Gram-positive and Gram negative Blood Culture (BC-GP, BC-GN) system (Nanosphere, USA) is a qualitative multiplexed automated nucleic acid in vitro diagnostic test for the direct identification of Gram-positive and Gram negative bacteria and their genetic resistance markers.
- Verigene BC-GP and BC-GN identifiable targets are show in **Table1 and Table 2, respectively**

Table 1: Verigene BC-GP Identifiable Targets:

Gram-Positive Blood Culture (BC – GP) Tests :			
Genus	Staphylococcus Spp. Streptococcus Spp. Micrococcus Spp. Listeria Spp.	Species	S. aureus S. epidermidis S. lugdumesis
			S. pneumoniae S. anginosus. Group S. agalaticae
Resistance	Mec A. Van A. Van B.		S. pyogenes Enterococcus faecalis Enterococcus faecium

NB. Of the staphylococci only *S.aures, S. epidermidis* and *S. lugdumesis* can be identified as the other staphylococci are not present in the data base.

Table 2: Verigene BC-GP Identifiable Targets:

Targets	Organism/Gene	
Bacterial Targets	*Acinetobacter* spp.	
	Citrobacter spp.	
	Enterobacter spp.	
	Proteus spp.	
	E. coli	
	Klebsiella pneumoniae	
	Klebsiella oxytoca	
	Pseudomonas aerogenes	
	Serratia marcescens	
Resistance Marker	CTX-M	VIM
	KPC	IMP
	NDM	OXA (48/23/40/58)

OBJECTIVES

- To evaluate the performance of Verigene (BC-GP and BC-GN) nucleic acid test for the direct identification of Gram-positive and Gram-negative bacteria from positive blood culture bottles in comparison with Gene–Xpert system (Cephide, USA) for Gram-positive bacteria and with the conventional culture technique for both Gram-positive and Gram-negative bacteria.
- To evaluate the performance of Verigene (BC-GP) and (BC-GN) for the detection of resistant markers directly from positive blood culture bottles in comparison with conventional culture technique.

MATERIALS AND METHODS

- All the demographic data including age, sex, patient location, underlying clinical condition, clinical and laboratory data suggesting sepsis, initial empirical therapy, adjusted therapy and outcome of the patients were collected.
- **For Gram-positive bacteria:**
  - All blood culture bottles (Bactec, Bekton Dickinson, USA) showing Gram-positive cocci by Gram stain were processed in:
    - Verigene for BC-GP according to the manufacturer's instructions
    - GeneXpert (Cepheid, USA) for BC-GP (only for Gram-positive cocci in clusters)
  - All the positive blood culture bottles were simultaneously cultured by conventional methods for both ID as well as susceptibility using Vitek II, and Vitek MS (Biomerioux, France)

- **For Gram-negative bacteria:**
  - All blood culture bottles showing Gram-negative bacilli by Gram stain were processed in:
    - Verigene for BC-GN according to the manufacturer's instructions
  - All the positive blood culture bottles were simultaneously cultured by conventional methods for both ID as well as susceptibility using Vitek II, and Vitek MS
    - A total of 11 QC strains of different *Streptococcus* spp. were included in the evaluation

Table 3: Comparison between results of Verigene and conventional culture of Gram-positive bacteria.

Gram-positive	Virigene	Conventional culture
Staphylococcus aureus	16	16
S.epidermidis	19	17
S.homonis	0	1
S.hemolyticus	0	3
Other Staphylococci	9	6
Enterococcus fecalis	9	9
Enterococcus fecium	4	4
Streptococcus pneumoniae	2	2
Streptococcus mitis	1	2
Streptococcus spp.	2	1
Micrococcus spp.	1	0

Table 4: Comparison between results of Verigene and conventional culture for Gram-negative bacteria

Gram negative	Verigene	Conventional culture	% Concordance
E.coli	24	24	100
Acinitobacter spp.	15	15	100
Klebsiella pneumoniae	8	8	100
Pseudomonas aeruginosa	7	7	100
Pseudomonas oryzihabitans	1	1	100
Enterobacter spp.	2	2	100
Proteus spp.	1	1	100
Serratia marcescens	1	1	100

RESULTS

A. Gram-positive bacteria:

- A total of 63 patients with positive blood culture for Gram-positive cocci were included in the evaluation
- The comparison between results of Verigene and conventional culture method for the 63 patients is shown in **Table 3**
- All the 11 QC strains were correctly identified by Verigene
- All *Staphylococcus* spp. Were correctly identified by Verigene and were in concordance with GeneXpert.
- Verigene correctly detected MecA gene in all coagulase-negative staphylococci but failed to detect it in two isolates of *S. aureus.*
- Verigene correctly identified 11 MSSA but wrongly identified two *S. aureus* isolates as MRSA.
- Verigene failed to detect VAN A and VAN B in two of the four *Enterococcus faecium* isolates

B. Gram-negative bacteria:

- A total of 63 patients with positive blood culture for Gram-negative bacilli were included in the evaluation
- The comparison between results of Verigene and conventional culture method for the 63 patients is shown in **Table 4**
- Verigene failed to detect three *Stenotrophomonas maltophilia* as the bacteria is not in the database
- Verigene correctly detected all ESBL-producing *Enterobacteriacae* but failed to detect resistance markers of the three MDR-*Psuedomonas aeruginosa* and 5 of the 6 *Acinitobacter baumanii* isolates.

C. Impact of rapid identification of Gram-positive and Gram-negative bacteria by Verigene on the modification of the empirical antibiotic therapy is shown in **Table 5** and **Table 6,** respectively

Table 5: Impact of rapid identification of Gram-positive bacteria on the modification of the empirical antibiotic therapy

Gram positive bacteria	De-escalate	Escalate	Continue same antibiotic	Stop antibiotic
Staphylococcus spp.	11	2	16	14
Enterococcus spp.	0	0	13	0
Streptococcus pneumoniae	0	0	2	0
Streptococcus mitis	0	0	0	1

Table 6: Impact of rapid identification of Gram-negative bacteria on the modification of the empirical antibiotic therapy

Gram- negative bacteria	De-escalate	Escalate	Continue same antibiotic
Enterobacteriacae	2	11	21
Pseudomonas aeruginosa	0	3	4
Acinitobacter spp.	0	6	9

CONCLUSION

- Verigene BC can be used for the direct and rapid identification of Gram-positive and Gram negative bacteria and their resistance markers directly from a blood culture bottles.
- The turn around time (TAT) for both the ID and detection of resistance markers is 4 hrs compared to 48 hrs using conventional culture techniques.
- Modification of empirical therapy is feasible following the rapid identification of bacterial isolates together with their resistance markers directly from positive blood cultures
- Rapid molecular methods used to identify both Gram-positive and Gram-negative bacteria directly from positive blood culture bottles in septic patients greatly helps the implementation of antimicrobial stewardship programs in the process to encourage rational use of antimicrobial agents with subsequent reduction in antibiotic resistance as well as cost.