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COMPARISON OF BD PHOENIX AUTOMATED SYSTEM WITH CONVENTIONAL METHODS FOR IDENTIFICATION & SUSCEPTIBILITY TESTING OF COMMON BACTERIA.

Microbiology

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ABSTRACT

Background- As hospitals face the continuing challenge of treating sicker patients, the burden falls to clinical microbiology laboratories to provide accurate and rapid identification and susceptibility of the pathogens recovered. To accomplish this goal, many laboratories rely upon automated systems. The objective of this study was to evaluate the performance of the Phoenix Automated Microbiology System (BD Biosciences, USA) system for identification and antimicrobial susceptibility of pathogens from clinical specimens vis-à-vis conventional method.

Methodology- A total of 81 isolates, 44 GNB and 37 GPC were tested by three types of phoenix panels for gram-negative bacilli (GNB), gram-positive cocci (GPC-*Staphylocooci and Enterococci*) and streptococci. The results were compared with conventional microbiological methods. To resolve discrepancy, tests were repeated by both methods.

Results- The overall levels of agreement till genus- and species-level identifications for GNB, GPC (*Staphylocooci and Enterococci*) and *Streptococci* (n=13) were 90.4% and 94.7%, 91.6 and 84.6%, 84.6% and 75% respectively. There were 16 discrepant identification results by phoenix (GNB- 10, GPC- 6).All salmonella were identified only till genus level by Phoenix while all 3 vibrios were incorrectly identified as aeromonas species.

For AST results, very major Discrepancy VMD (false susceptible result with phoenix), major Discrepancy MD (false resistant with phoenix) and minor Discrepancy mD rates were calculated. For GNB susceptibility, VMD=2.8%, MD=4.4%, mD=1.1% were seen. For Gram-positive cocci VMD=1.5%, MD=3.1%, mD=2.3%. The mean duration of test for phoenix was 14.7 hours as compared to19 hours by conventional methods.

Conclusion-The phoenix performed favorably and faster than traditional methods for ID and AST of common pathogens with exception of identification of Vibrio spp.

KEYWORDS

Comparison, BD PHOENIX, Conventional method

INTRODUCTION

The epidemiology of infectious diseases is rapidly changing. There is increase in number of patients who are seeking treatment in critical care facilities. So Clinical microbiologists are greatly concerned about providing rapid and accurate laboratory reports.[1]Conventional microbiological methods for identification and sensitivity are time consuming. Also, using battery of biochemical tests to differentiate between species of bacteria is cumbersome. Kirby bauer disk diffusion technique is used for performing antimicrobial susceptibility testing in many laboratories. However there are limitations to this technique while testing many antimicrobials like vancomycin, colistin, ciprofloxacin where Minimum inhibitory concentrations (MIC) are recommended by standard guidelines. Clinicians are also becoming aware about MICs as they increase the success of antimicrobial therapy especially in critical care settings. Automated methods are user friendly and provide rapid results.The advantages of automated methods are they provide MICs with reduced turn around time (TAT) of reporting.

We investigated the ability of the Phoenix system to accurately perform ID and AST of clinical isolates in the department of microbiology at a tertiary care hospital.

Methodology-

In this study, A total of 81 isolates, 44 Gram-negative bacilli (GNB) and 37 Gram-positive cocci (GPC) were tested by 3 types of phoenix panels like NMICID55 for GNB ,PMICID55 for *Staphylocooci, Enterococci* and SMIC 9 for *Streptococci* The processing for phoenix was done as per instructions from manufacturer. The processing by conventional microbiological methods was done as per standard protocol.[2] Antimicrobial susceptibility testing (AMST) was done by Kirby bauer disk diffusion method as per CLSI guidelines.[3]

The data analysis was done on Excel spreadsheets. For identification of bacteria, genus level and species level agreement between the two methods was calculated. For AMST, very major Discrepancy (VMD), major Discrepancy (MD) and minor Discrepancy (mD) were calculated for phoenix. VMD- a false-susceptible result with the Phoenix system. MD- false-resistant result with the Phoenix system. mD- one system reported an intermediate result, while the other method reported a resistant or susceptible result. The number of resistant strains was used as the denominator for the calculation of the VMD rates. For the calculation of MD rates, the number of susceptible strains was used as the denominator. Categorial agreement (CA) was calculated for both the methods under comparison as the conventional method (disk diffusion) as per CLSI is based on interpretations as S, I and R[4].

Discrepancy resolution- For resolution of discrepant identification results between the two systems, testing was repeated by both methods. Biochemical testing was accepted as the "gold standard." For susceptibility testing, both methods were likewise repeated for any organism-drug discrepancy between the two systems that resulted in MDs and VMDs.

Results-

A total of 81 clinically significant isolates, gram positive (n=37) & gram negative(n=44) bacterial pathogens were included in this study. For comparison for identification of bacteria by conventional method and phoenix genus level and species level agreement was calculated.

TABLE 1. Comparison of GNB by conventional methods and the

Phoenix (n=44)

Genus & species	Conventional method	Phoenix ID system	% Agreement
E.coli	09	08	**89.9**
Pseudomonas aeruginosa	05	05	**100%**
Proteus mirabilis	03	03	**100%**
Klebsiella pneumoniae	03	03	**100%**
Klebsiella oxytoca	02	02	**100%**
Acinetobacter baumanii	06	05	**90%**
Acinetobacter baumanii/calcoceticus complex	0	01	**0%**
Acinetobacter spp	06	06	**100%**
Citrobacter freundii	01	01	**100%**
Serratia marcescens	2	2	**100%**
Providentia rettgeri	1	1	**100%**
Enterobacter spp	3	3	**100%**
Enterobacter clocae	2	2	**100%**
Enterobacter amnigenus group	0	1	**0%**
Pantoea agglomerans	0	1	**0%**
Stenotrophomonas maltophila	0	1	**0%**
Salmonella spp	02	02	**100%**
Salmonella paraA	01	0	**0%**
Salmonella paraB	01	0	**0%**
Vibrio spp	03	0	**0%**
TOTAL genus level	**42**	**38**	**90.4%**
Total species level	38	36	94.7%

There was 90.4% genus level agreement and 94.7% species level agreement while identifying GNB by both methods.There was 100% agreement for genera like *Klebsiella, Pseudomonas, Acinetobacter, Salmonella* and *Enterobacter* .Phoenix could not identify 3 strains of Vibrio which conventional method could do. The species level agreement for GNB was 94.7%. The phoenix identified salmonella serotypes as salmonella species which were identified till species level by conventional methods such as serotyping.

Table 2- Comparison of GPC by conventional methods and the Phoenix (n=24)

Genus & species	Conventional method	Phoenix ID system	% Agreement
Staphylococcus genus	10	10	100%
S.aureus	6	6	100%
CONS	4	4	100%
S.epidermidis	0	2	0%
S.hemolyticus	0	1	0%
S.cohnii subspp urealyticum	0	1	0%
Enterococcus genus	14	12	86%
E.faecalis	7	6	86%
E.faecium	5	6	83%
TOTAL genus level	**24**	**22**	**91.7%**
Total species level	**22**	**26**	**84.6%**

Table 2 shows that there was 91.7% genus level agreement while identifying Staphylococci and enterococci. The species level identification was 84.6% as conventional methods failed to identify 4 strains of Coagulase negative staphylococci till species level.

Table 3- Comparison of streptococci by conventional methods and the Phoenix (n=13)

Genus & species	Conventional method	Phoenix ID system	% Agreement
Streptococcus genus	13	11	84.60%
S.pyogenes	2	1	50%
S.pneumoniae	4	2	50%
Other Streptococcus spp	7	5	71.40%
TOTAL genus level	**13**	**11**	**84.6%**
Total species level	**6**	**8**	**75.0%**

Genus level and species level agreement for identifying streptococci was 84.6% and 75% respectively. The phoenix could identify 2 more strains of streptococci than conventional method.

TABLE 4. –Identification of discrepancies between conventional method and the Phoenix system

Overall there were 18 discrepancies (22%) for identification either at genus or species level. They were as shown in table no. 4.

Sr no	Conventional method	Phoenix system ID
1	*E.coli*	*Pantoea agglomerans*
2	*Enterobacter spp*	*Enterobacter amnigenus group*
3	*Nonfermenter*	*Stenotrophomonas maltophila*
4	*Acinetobacterspp*	*Acinetobacter baumanii/calcoceticus complex*
5	*Salmonella para A*	*Salmonella spp*
6	*Salmonella para B*	*Salmonella spp*
7	*Nonfermenter*	*Burkholderia cepacia complex*
8	*Vibrio cholera Inaba*	*Aeromonas veronii*
9	*Vibrio cholera NAG*	*Aeromonas hydrophilla*
10	*Vibrio cholera ogawa*	*Aeromonas testesteronii*
11	*Enterococcus spp*	*Aerococcus viridans*
12	*Enterococcus faecium*	*Bacillus caogulans*
13	*Enterococcus faecalis*	*Enterococcus faecium*
14	*Streptococcus pneumoniae*	*Streptococcus acidominimus*
15	*Streptococcus pyogenes*	*Streptococcus mitis*
16	*Streptococcus spp.*	*Streptococcus acidominimus*
17	*Streptococcus spp.*	*Streptococcus sanguinis*
18	*Streptococcus spp.*	*Streptococcus intermedius*

TABLE 5. Susceptibility testing results for GNB (n=44)

Antibiotic	Total no. of isolates tested	By conventional method			By B.D.phoenix			CA	No. (%) of isolates with		
		S	I	R	S	I	R	%	VMD	MD	mD
Amikacin	39	23	2	14	23	1	15	95	0	01(2.5%)	01(2.5%)
Gentamicin	39	18	1	20	16	1	23	92.3	0	03 (7.7%)	0
Imipenem	39	32	0	7	31	0	8	97.5	0	1(2.5%)	0
Cefoxitin	37	11	1	25	10	1	26	97.3	0	1(2.7%)	0
Ceftazidime	22	4	0	18	4	0	18	100	0	0	0
Ciprofloxacin	38	16	1	21	17	1	20	97.4	1(2.6%)	0	0
Cefotaxime	38	25	0	13	13	1	24	68.5	0	11(28.9%)	1(2.6%)
Cefepime	39	13	1	25	23	1	15	74.6	10 (25.4%)	0	0
Piperacillin /tazobactum	37	28	1	8	26	2	9	94.6	0	1(2.7%)	1(2.7%)
Colistin	21	14	0	7	13	1	7	95.3	0	0	1(4.7%)
Co-trimoxazole	39	19	0	20	20	0	19	97.5	1(2.5%)	0	0
Tetracycline	38	16	0	22	14	1	23	94.6	0	1(2.7%)	1(2.7%)
Total	426	219	7	200	210	10	206	91.7	12(2.8%)	19(4.4%)	5(1.1%)

While comparing the results of susceptibility by two methods, VMD, MD and mD was calculated as mentioned in methodology. In the present study (table 5) categorical agreement of 91.7% between the two methods was seen.There was 2.8% VMD, 4.4% MD and 1.1% mD for susceptibility of GNB. VMD was mainly observed for cefepime, MD for cefotaxime and mD for cefotaxime, piperacillin/tazobactum, tetracycline and colistin (one case each).

Table 6- Susceptibility testing results for staphylocci and enterococci (n=24)

Antibiotic	Total no. of isolates tested	By conventional method			By B.D.phoenix			CA	No. (%) of isolates with		
		S	I	R	S	I	R	%	VMD	MD	mD
Gentamicin	22	8	0	14	7	0	15	95.4	0	1(4.5%)	0
Cefoxitin	22	4	0	18	3	0	19	95.4	0	1(4.5%)	0
Ciprofloxacin	22	7	2	13	7	1	14	91.0	0	1(4.5%)	1(4.5%)
Co-trimaxazole	22	10	0	12	7	0	15	86.4	0	3(13.6%)	0
Penicillin	16	4	0	12	3	0	13	93.8	0	1(6.2%)	0
Oxacillin	22	3	0	19	3	0	19	100	0	0	0
Vancomycin	22	13	2	7	13	2	7	100	0	0	0
Teicoplanin	22	15	0	7	17	0	5	91.0	2(9.0%)	0	0
Linezolid	22	21	0	1	19	3	0	86.4	0	0	3(13.6%)
Erythromycin	22	6	2	14	6	1	15	91.0	0	1(4.5%)	1(4.5%)
Clindamycin	22	8	0	14	8	0	14	100	0	0	0
Tetracycline	22	12	1	9	14	0	8	86.5	2(9.0%)	0	1(4.5%)
Total	**258**	**111**	**7**	**140**	**107**	**7**	**144**	**93.1**	**4(1.5%)**	**8(3.1%)**	**6(2.3%)**

For Staphylococci and enterococci an overall CA of 93.1% with 1.5% VMD, 3.1% MD and 2.3% mD was observed.VMD was observed for teicoplanin and tetracycline (9% each). MD was observed mainly for co-trimoxazole (13.6%) and mD was observed for Linezolid (13.6%). For vancomycin, oxacillin and clindamycin, 100% CA for found.

Turn around time-The mean duration of ID by phoenix was 6-8 hours and that of sensitivity was **14.7 hours** as compared to18-19 hours by conventional methods.

Costing – One of the limitation of the automated method is cost. Conventional methods are cost effective as compared to automated. By automated method cost per test goes about Rs 500 as compared to Rs 200-250 of manual method.

Discussion-

In the present study we compared performance of phoenix system with that of conventional methods .A total of 81 isolates comprising of 44 GNB and 37 GPC were tested by both methods and results were compared. For GNB, agreement for genus level identification between two methods was 90.4% and species level identification agreement was 94.7%. In a study by Carrol et al 250 GNB were included in the study with genus level agreement of 95.6% and species level agreement of 94.4% [5]. In another study by Donay et al comprising of 130 GNB strains, genus level identification agreement rate was 90.6% while that of species level agreement was 93.3%.[6]

These findings are consistent with findings of our study. In the present study there was 100% agreement for genera like *Klebsiella, Pseudomonas, Acinetobacter, Salmonella* and *Enterobacter* with exception of escherechia which showed 89.9% agreement. One strain of *E.coli* was identified by phoenix as *Pantoea agglomerans*. In a study by Maria et al Out of 494 strains tested, a concordant ID to the species level was obtained in 98.6% The various species of *Enterobacte riaceae* and of NFGNRs showed concordant species results of 98.4% and 99.1%, respectively.[7] In a study by Dallas et al there was 95% concordance rate for GNB identification between phoenix n other system.In their study discorant result was seen with one isolate of *E.coli* and other isolate of *klebsiella pneumoniae* which were falsely identified as *Citrobacter freundii* and *Klebsiella oxytoca* respec tively.[7] In the present study conventional methods could not identify the non-fermenter GNB (NFRGNB) till species level as there are limitations to the number of tests which can be used manually due to lack of reagents and non-availability of time.

One species of NFRGNB was correctly identified by phoenix as *Stenotrophomonas maltophila*. One isolate of *Acinetobacter spp* was identified till species level as *Acinetobacter calcoceticus* complex by phoenix.Hence we interprete that automated methods are a must especially for identification of NFRGNB. Stefaniuk et al. [8] observed a 96.0% accuracy for gram-negative nonfermenters and 92.5% for members of the *Enterobacteriaceae* family in a study comparing the Phoenix system to conventional identification methods using 174 gram-negative strains, including eight different species of *Enteroba cteriaceae* and three different species of nonfermentative bacteria. *Endimiani et al.* [9] evaluated 136 strains of seven species of nonferm entative organisms and reported an overall 95.6% comparability with the ATB ID32GN test (bio- Me´rieux,) and a cumulative agreement of 97.1% when two isolates, concordant at the genus level, Brisse et al. [10] evaluated the Phoenix and VITEK 2 systems for the identification of 134 strains of *Burkholderia cepacia* or closely related nonfer mentative bacteria using genetic/molecular typing as a reference, the Phoenix system correctly identified 81.0% of isolates of the most clinically relevant strain, B. cepacia genomovar III.

However Phoenix could not identify 3 isolates of Vibrio which it identified as aeromonas which is a very closely related genus to Vibrio.There was 100% agreement for genus salmonella but for speciation conventional serotyping could identify till serovar level which phoenix could not.In the study of Caroll et al 6 strains of salmonellae were not speciated by phoenix similar tto our study.[5]

In the present study, there was 100% agreement for identification of genus *Staphylococcus* but 86% agreement for genus *Enterococcus*. The 2 isolates of enterococci were identified by phoenix as aerococcus and bacillus coagulans.Here for aerococcus viridians there could be correct identification by phoenix as enterococcus and aerococcus are very closely related genera which are very difficult to separate by conventional biochemical tests.Fahr et al [11] noted Concordant IDs of 97.1, 98.9, and 100% were observed for staphylococci, enterococci, and streptococci, respectively for phoenix with other routine ID systems. Salomon et al. [12] could demonstrate the discriminatory power of the different substrate classes used in the Phoenix system with more than 1000 GPC. This resulted in a list of approximately 100 gram-positive species (taxon list) which can be identified by the Phoenix system. Marco et al.[13] compared 136 gram-positive cocci with the Phoenix instrument and the MicroScan Walk-Away-40 (Dade-MicroScan, W. Sacramento, Calif.) and reported a concordance with this MicroScan system of 98.5%

When comparing the performance of the Phoenix instrument with the VITEK 2 system Gross et al.[14] investigated 400 staphylococcal strains and 121 Enterococcus spp. Out of 520 gram-positive strains tested, 498 gave similar ID results in both systems . Liu et al,[15] has reported correct ID rate of 97.4% for 158 isolates of Staphylococci and Enterococci for phoenix with API ID system.

In the present study genus level agreement rate for *Streptococcus* spp was 84.6% while species level agreement was 75%. This poor agreement rate could be because we used SMIC ID-9 panel in our study. Bringate et al [16] has reported good concordance with API ID by using Phoenix SMIC-ID 2 panels. In their study the authors have reported, correct ID rate of 93.8% for *Streptococcu spp* with excellent results for *Streptococcus pneumoniae (100%)* and Beta hemolytic streptococci (49/50). However 83.9% correct ID rate was for *Viridans streptococci.*

In the present study AST results were compared between two systems for 44 GNB and 24 GPC. AST results were not analysed for *Streptococcus spp* as we could not speciate 7 out of 13 isolates and concordance rate between two methods was also less. We observed an overall categorical agreement (CA) of 91.7%. with Discrepancy of 2.8% VMD, 4.4% MD and 1.1% mD for susceptibility of GNB. For ceftazidime 100% CA was found.VMD was mainly observed for

cefepime (25.4%), MD for cefotaxime (28.9%) and mD for cefotaxime (2.6%), piperacillin/tazobactum (2.7%), tetracycline (2.7%) and colistin (4.7%). Menozii et al [a] observed that overall CA for AST of GNB was above 94%. VMDs were only found for ampicillin and ticarcillin (1.7% and 0.4%, respectively); the MD rates in this drug class ranged from 1.4% to 6.8%. Caroll et al observed over all CA of 97.9% with VMD of 0.38% , MD of 0.33% and mD of 1.8% [5].

In the present study, comparison of AST results of Staphylococci and Enterococci showed an overall CA of 93.1% with 1.5% VMD, 3.1% MD and 2.3% mD .VMD was observed for teicoplanin and tetracycline (9% each). MD was observed mainly for co-trimoxazole (13.6%) and mE was observed for Linezolid (13.6%).

In a study by Fahr et al,[11] category agreement was 97.3%; and the VMD, MD, and mD rates were 1.2, 1.9, and 1.3%, respectively. In the present study we observed CA of 93.8% to penicillin which is comparable to CA of 85% to penicillin reported by Wile's et al [q] Marco et al.[m] also reported a Phoenix CA of 93.7% in comparison to the MicroScan system result for penicillin, and Gross et al.[14] found a 99.1% Phoenix CA in comparison to the VITEK 2 system.

In the present study phoenix CA of 95.4% was observed for cefoxitin with ME of 4.5%.That means there was a good agreement for detection of MRSA between both the systems.

Vancomycin is a drug of choice for treating serious infections by staphylococci and enterococci .[18] In the present study for vancomycin, oxacillin and clindamycin, 100% CA for found. Fahr et al [11] also noted 99.3% CA to vancomycin consistent with our study . But Fahr et al [11] reported 100% CA to teicoplanin which is not matching with our study.

In conclusion BD PHOENIX ID system appears to be a reliable tool with a potential of routine use in clinical microbiology laboratory for identification and antimicrobial susceptibility testing of bacteria.

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