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Research Article

Ease with VITEK 2 systems, biomerieux in identification of non-lactose fermenting bacteria including their antibiotic drug susceptibility: our experience

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ABSTRACT

Background: Nonfermenters are being isolated from various clinical specimens. Although frequently considered as contaminants, the pathogenic potential has been proved beyond doubt by their frequent isolation from clinical material and their association with disease. In the recent years due to the liberal and empirical use of antibiotics, Non Fermenting Gram Negative Bacilli (NFGNB) have emerged as important health care associated pathogens. Several automated systems are available for the identification and susceptibility of the clinically important bacteria. In this study we have evaluated the ease of the VITEK-2 Compact in identifying the NFGNB along with its antibiotic sensitivity.

Methods: A total of 186 strains which grew in culture from various clinical specimens isolated at the Clinical Microbiology Laboratory, NRI General Hospital, Chinakakani, Guntur District, A.P. during the period from January 2015 to June 2015 were included in the study. The Vitek-2 Compact machine was validated using the standard strains as per the manufacturer's instructions. The isolates were processed as per the Manufacturer's instructions for Identification and Antimicrobial Sensitivity Testing (AST).

Results: Out of the 186 strains, 50 strains were isolated from tracheal aspirate, 47 from pus/wound infections, 43 from blood cultures, 25 from urine, 20 from sputum and one from central line tip. The VITEK-2 compact system identified all the strains with a level of 95-99% probability. Most of the strains were identified as Pseudomonas aeruginosa followed by Acientobacter baumannii. Pseudomonas aeruginosa strains were most susceptible to Meropenem (72%) and least susceptible to Cefuroxime and Trimethoprim/Sulfamethoxazole (0%) while Sphingomonas paucimobilis showed resistance to all the antibiotics tested.

Conclusions: Care in detection, evaluation of effective antibiotic options, and judicious use of antibiotics by instituting antibiotic policy for combination therapy and rigorous infection control measures will help us to fight against these multidrug resistant NFGNB during the effective management of patients.

Keywords: NFGNB, VITEK-2 Compact, Drug resistance, Identification

INTRODUCTION

Nonfermenters are being isolated from various clinical specimens. Although frequently considered as contaminants, the pathogenic potential has been proved beyond doubt by their frequent isolation from clinical material and their association with disease.[1,2] Non-Fermenting Gram Negative Bacilli (NFGNB) are known

to account for about 15% of all bacterial isolates from a clinical Microbiology Laboratory.[1,3,4,5]

In the recent years due to the liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogens.[1,3,6] Several automated systems are available for the identification and susceptibility of the clinically important bacteria.[7,8] More recently the new VITEK-2 Compact system (Biomerieux, France) has been introduced. The VITEK-2 compact system detects metabolic changes by fluorescence based methods which facilitate the identification of gram negative bacteria within 6 hours. This system monitors the kinetics of bacterial growth and calculates Minimum Inhibitory Concentrations (MIC) using a unique algorithm.[9]

METHODS

A total of 186 strains which grew in culture from various clinical specimens isolated at the Clinical Microbiology Laboratory, NRI General Hospital, Chinakakani, Guntur District, A.P. during the period from January 2015 to June 2015 were included in the study. Only Non-Lactose Fermenting (NLF) Gram Negative bacteria that grew well in MacConkey's agar were included.

Inoculum Preparation – From the isolated colonies grown on the media, a bacterial suspension was prepared in 3 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12x75 mm clear plastic (polystyrene) test tube. The turbidity of the suspension was adjusted to a McFarland standard of 0.5 with the help of a VITEK-2 DensiCheck instrument.[8] The time between the preparation of inoculum and filling of the card was always less than 30 min.

Identification with the VITEK-2 compact system was performed using a Gram Negative (GN) card according to the Manufacturer's instructions.[10] The 64 well plastic GN card contains 41 tests including 18 tests for sugar assimilation, 18 tests for sugar fermentation, 2 decarboxylase tests and 3 miscellaneous tests (for urease, utilization of malonate and tryptophan deaminase). The culture suspension was inoculated into the GN Card with the help of a vacuum device inside the filling chamber. The cards were later transferred into the loading chamber where the cards were sealed and were incubated in a rotating carousel at 37°C. Each loaded card was removed from the carousel for every 15 minutes, transported to the optical system for reaction readings and the returned to the carousel incubator until the next read time. Data was collected at 15-minute intervals during the entire incubation period.

Quality control

The Vitek-2 Compact machine was validated using the standard strains as per the manufacturer's instructions. *Acinetobacter baumanii* ATCC BAA-747, *Aeromonus hydrophila* ATCC 35654, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used. During the study period, the control strains were checked at regular intervals.

Antimicrobial susceptibility testing

Antimicrobial Susceptibility testing with the VITEK-2 compact system was performed using an AST N281 card according to the Manufacturer's instructions. The VITEK-2 AST N281 susceptibility card is intended for use with the VITEK-2 systems in clinical laboratories as an *in-vitro* test to determine the susceptibility of clinically significant aerobic gram negative bacilli to antimicrobial agents.[10,11] Antibiotics tested in AST N281 card included Levofloxacin, Gentamicin, Cefepime, Meropenem, Imipenem, Ticarcillin/Clavulinic acid, Doripenem, Ceftazidime, Cefoperazone/Sulbactum, Amikacin, Ciprofloxacin, Minocycline, Tigecycline, Colistin, Trimethoprim/Sulfomethoxazole (Cotrimoxazole), Cephotaxime, Piperacillin/Tazobactum, Cefuroxime, Ceftriaxone, Tobramycin.

The cards were filled with an inoculum (Prepared by transferring 200µL of culture suspension from the 0.5 McFarland culture suspension used for filling the identification cards into a fresh 3mL sterile saline solution obtaining a final turbidity of $8x10^6$ cfu/mL) in the filling chamber. The VITEK-2 System automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The VITEK-2 compact system subsequently corrects, where necessary for MIC's or clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations.[10,11]

RESULTS

We did not encounter any major technical problems using the VITEK-2 Compact instrument during evaluation. Quality control Strains were correctly identified to the species level in every instance, demonstrating the reliability as well as the reproducibility of the instrument.

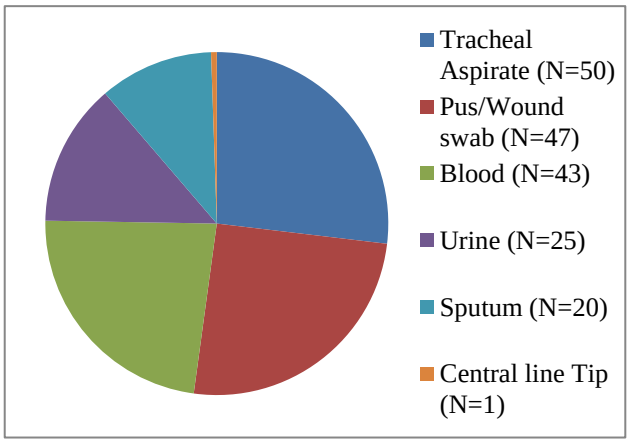

Figure 1: Pie Diagram showing the distribution of specimens used in the study.

A total of 186 strains collected from various clinical specimens were tested in VITEK-2 compact system. Out of the 186 strains, 50 strains were isolated from tracheal aspirate, 47 from pus/wound infections, 43 from blood cultures, 25 from urine, 20 from sputum and one from central line tip (Figure 1). The VITEK-2 compact system identified all the strains with a level of 95-99% probability (Table 1). Two strains (1.8%) of *Pseudomonas aeruginosa* and 2 strains (2.8%) of *Acinetobacter baumannii* were identified with a low level discrimination at 85-90% probability. No strain remained unidentified during our study. Discrepant results were resolved by doing additional biochemical tests. *Pseudomonas aeruginosa* (57.5%) was the most common strain identified followed by *Acinetobacter baumanii* (38.1%).

Pseudomonas aeruginosa strains were most susceptible to Meropenem (72%) and least susceptible to Cefuroxime and Trimethoprim/Sulfamethoxazole (0%). Strains of Acinetobacter spp. were most susceptible to Colistin (93.7% - 100%) and lest susceptible to Cefuroxime and Trimethoprim/Sulfamethoxazole (0%). *Burkholderia cepacia* were found to be sensitive to Levofloxacin, Meropenem, Minocycline, Trimethoprim/Sulfamethoxazole, and Ceftriaxone (100%) while *Sphingomonas paucimobilis* showed resistance to all the antibiotics tested.

Table 1: Characteristics of the patients with prevalence of dyslipidemia (n = 285).

Organism Identified		No of strains				Total number of strains tested
Genus	**species**	**Correctly Identified**	**Identified with low discrimination**	**Not identified**	**Mis Identified**	
Pseudomonas	*aeruginosa*	105	2 (1.86%)	0	0	107
Acinetobacter	*baumannii*	69	2 (2.81%)	0	0	74
	calcoaceticus	2	0	0	0	
	hydrophila	1	0	0	0	
Burkholderia	*cepacia*	4	0	0	0	4
Sphingomonas	*paucimobilis*	1	0	0	0	1

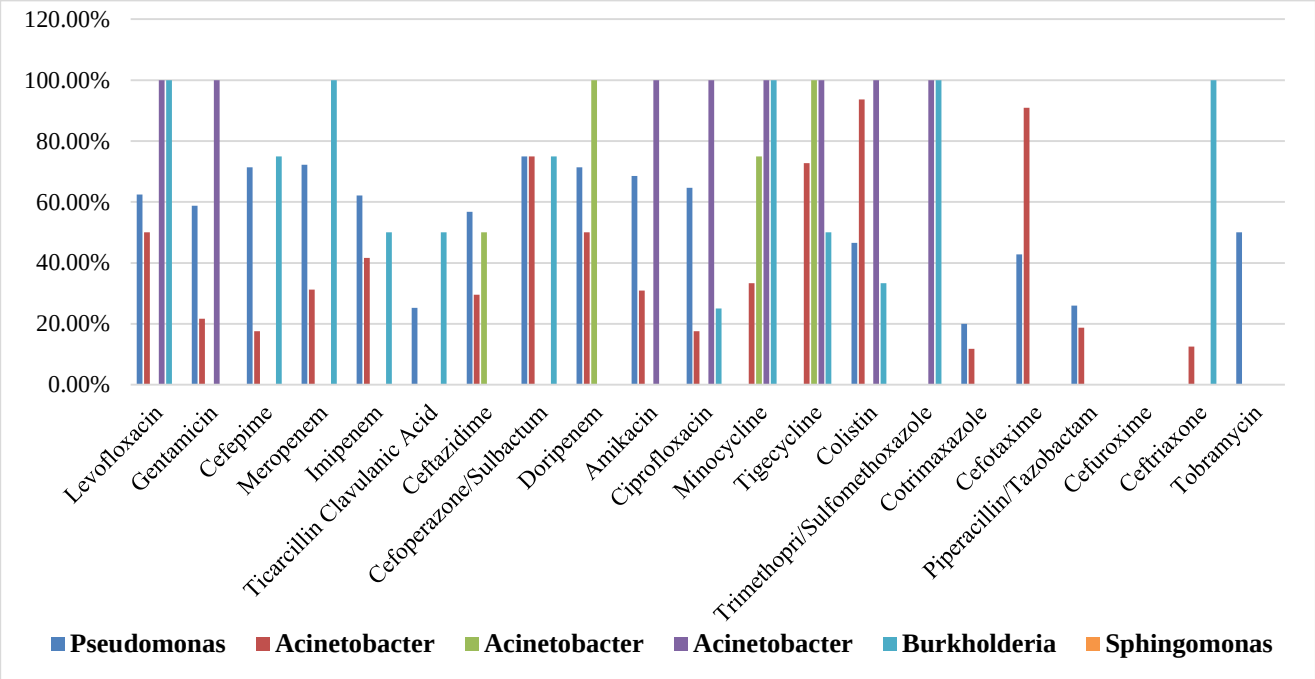

Figure 2: Diagram showing the percentage sensitivities of the Non Fermenting gram negative bacilli towards various antibiotics.

DISCUSSION

NFGNB that were previously considered to be contaminants have now emerged as important nosocomial pathogens.[5,12] *P. aeruginosa* and *Acinetobacter* species are known to be common among them.[13] The VITEK-2 compact system combines several advantages that may be of clinical interest for routine testing of gram negative rods isolated from clinical samples like rapid identification, a simple methodology, a high level of automation and taxonomically updated databases.

Earlier studies[13] have proved an efficiency of VITEK-2 System with 85.5% probability of accurate identification of strains but in our study we found to achieve 90-95% probability of identification. A study conducted by Joyanes et al reported to have discrepant results with *P. aeruginosa* (7.5%) and *A. baumannii* (24%) but we had a very low level of discrepant results with *P. aeruginosa* (1.8%) and with *A. baumannii* (2.8%).[9] This may be due to the difference in increasing levels of capacity and automation.[8]

In our study, we compared the antibiotic susceptibility patterns of the NFGNB using VITEK-2 Compact with those of the studies conducted manually using the Disk Diffusion Method. It was intended to know the resistance patterns of NFGNB as they have become a major problem in hospitals causing nosocomial infections. *Pseudmonas aeruginosa* isolates in our study were susceptible to Cefoperazone/Sulbactum, Meropenem, Doripenem, Cefepime, Amikacin and Levofloxacin which were similar to the earlier studies conducted by Kalidas Rit et al but were contradictory to the findings in the study conducted by Taneja N, et al and Kumari HB et al.[3,14,15]

The strains of *Acinetobacter* species showed high rate of resistance to Ciprofloxacin, Amikacin & Ceftazidime in an earlier study by Kumari HB et al which were in correlation to the results found in our study.[15] Susceptibility patterns may be altered due to resistant transfer and mutant selection from indiscriminate and excessive use of antibiotics.[3,16,17]

We evaluated the results of susceptibility testing with the VITEK-2 system taking in to account the clinical categories defined by the expert system in order to simulate, as much as possible the performance of the system in the routine work of clinical laboratory.

CONCLUSION

The VITEK -2 compact system identified a significant number of NFGNB along with their antibiotic susceptibility patterns with in a time period of 8 to 16hrs, which may be clinically relevant, because rapid reporting of microbiology results to physicians has been shown to significantly reduce the mortality rate and earlier initiation of appropriate antimicrobial therapy, a shorter hospital stay. So, the proper identification of NFGNB up to the species level along with its susceptibility patterns is important for proper management of the infection caused by them.

Care in detection, evaluation of effective antibiotic options, and judicious use of antibiotics by instituting antibiotic policy for combination therapy and rigorous infection control measures will help us to fight against these multidrug resistant NFGNB during the effective management of patients.

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REFERENCES

1. Gokale SK, Metgud SC. Characterization and antibiotic sensitivity pattern of nonfermenting gram negative bacilli from various clinical samples in a teritiary care hospital, BELGAUM-JPBMS. 2012;17(14).
2. Aliga L, Mediavilla JD, Cobo F. A clinical index predicting mortality with pseudomonas aeruginosa bacteremia. J Med Microbiol. 2002;51(7):615-9.
3. Rit K, Nag F, Raj HJ, Maity PK. Prevalence and susceptibility profiles of Nonfermentative Gram-negative Bacilli infection in a Tertiary care Hospital of Eastern India. Indian Journal of Clinical practice. 2013;24(5).
4. Patel PH. Prevalence of non-fermenting gram negative bacilli infection in tertiary care Hospital in Ahmedabad, Gujarat. Indian journal of Basic Applied Medical Research. 2013;2(6):608-13.
5. Kirthilaxmi K, Benachinmardi. Prevalence of nonfermenting Gram negative bacilli and their in vitro susceptibility pattern at a teritiary care teaching Hospital. Journal of scientific society. 2014;41(3).
6. Bergogne - Berezin E, Towner KJ. Acinetobacer spp as Nosocomial pathogens: Microbiological, Clinical and Epidemiological features. Clin Microbial Rev. 1996;9(2):148-65.
7. Stager CE, Davis JR. Automated Systems for identification of microorganisms. Clin Microbiolol. 1992:302-27.
8. David H. Pincus Biomerieux, inc Hazelwood, Mo, USA; Microbial identification using the Biomerieux VITEK- 2 System.

9. Joyanes P, del Carmen Conejo M, Martinez-Martinez L, Perea EJ. Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of nonfermenting gram-negative rods frequently isolated from clinical samples. J Clin Microbiol. 2001;39:3247-53.
10. Biomeriux VITEK-2 Compact ref Manual – Ref-414532.
11. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7 A5. Normal committee for clinical laboratory standards. Wayne, Pa. 2000.
12. Bruno D, Nishano MK, Prior WN, Remus PR, Do Cormo AA. Prevalence of Gram negative nonfermenters Patients in proto Alegre – RS. Journal Brasileiro de Patologia e Medina Laboratorial. 2011;47:529-34.
13. Funke G, Monnet D, deBernardis C, von Graevenitz A, Freney J Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. J Clin Microbiol. 1998;36:1948-52.
14. Taneja N, Maharwal S, Sharma M. Imipenem resistance in nonfermenters causing nosocomial urinary tract infections. Indian J Med Sci. 2003;57(7):294-9.
15. Kumari HB, Nagaratna S, Chandramukhi A. Antimicrobial resistance pattern among aerobic gram negative bacilli of lower respiratory tract specimens of intensive care unit patients in a neuro centre. Indian J Chest Dis Allied Sci. 2007;49(1):19-22.
16. Gilardi GL. Antimicrobial susceptibility as a diagnostic aid in the identification of nonfermenting gram negative bacteria. Appl Microbial. 1971;22(5):821-3.
17. Von Gravenitz A. Clinical Microbiology of unusual pseudomonas species. Prog Clin pathol. 1973;5:185-218.

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