British Microbiology Research Journal
14(2): 1-8, 2016, Article no.BMRJ.24999
ISSN: 2231-0886, NLM ID: 101608140

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Comparison of Automated System Vitek-2 with Conventional Methods, for Identification and Antibiotic Sensitivity in Gram Negative Organisms

Nayeem-u-din Wani[1*], Mohd Suhail[1], Dalip Kakru[1], Nargis Bali[2], Amrish Kohli[1] and Junaid Ahmad[1]

[1]Department of Clinical Microbiology, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, 190011, Kashmir, India.
[2]Department of Microbiology, Government Medical College, Srinagar 190010, Kashmir, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author NUDW designed the study, wrote the protocol. Authors MS, DK wrote the first draft of the manuscript managed literature searches, managed the analyses of the study. Authors NB, AK and JA performed the statistical analysis and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/24999
Editor(s):
(1) Gyanendra Singh, Gene Therapy & Louisiana Vaccine Center, School of Medicine, LSU Health Sciences Center, Louisiana, USA.
Reviewers:
(1) Andrea Cortegiani, University of Palermo, Italy.
(2) Enty Tjoa, Catholic Atma Jaya University, Indonesia.
(3) V. V. Shailaja, Niloufer Hospital Affiliated to Osmania Medical College, Hyderabad, India.
Complete Peer review History: http://sciencedomain.org/review-history/14138

Original Research Article

Received 12th February 2016
Accepted 1st April 2016
Published 12th April 2016

ABSTRACT

Background: Rapid bacterial identification and susceptibility testing improves patient therapy and outcome and decreases emergence of resistance. There is a need to provide rapid, efficient and accurate system for identification and antimicrobial susceptibility testing of pathogens. In this regard the automated identification/AST systems aid in rapid diagnosis/treatment of bacterial pathogens.
Aim: Comparison of automated system Vitek-2 with conventional methods, for identification and antibiotic sensitivity in Gram negative organisms.
Settings and Design: This was a prospective study conducted in the Department of Microbiology at SKIMS, Srinagar, for eight months.
Materials and Methods: A total of 135 non duplicate isolates of gram negative bacteria were

**Corresponding author: E-mail: drnayeemwani@gmail.com, Co-author: drmohmmadsuhail@gmail.com;*

included. Organisms were processed on the Vitek-2 system and by manual methods (ID/AST) for comparison.
Statistical Analysis Use: Descriptive statistics (frequency and percentage) was used.
Results and Conclusions: Vitek-2 misidentified 6 isolates of *S. typhi* as *E. coli*, *K. oxytoca* (3 isolates) misidentified as *K. pneumoniae*, *Citrobacter braakii* (1 isolate) falsely reported as *Citrobacter freundii*, *Acinetobacter baumannii* (2 isolates) misidentified as *Acinetobacter lwofii.* No minor error (mE), major error (ME) or very major error (VME); with 100% categorical agreement (CA) was seen with ampicillin+sulbactam, piperacillin+tazobactam, ceftriaxone, cefepime, gentamicin, amikacin, levofloxacin, meropenem, colistin, co-trimoxazole, tetracycline, carbenicillin and tobramycin for various Gram negative organisms tested.however errors were seen with *E. coli* for ampicillin and imipenem. Likewise with *K. pneumoniae*, errors were seen for a ME for ciprofloxacin and imipenem. Also with *P. aeruginosa*, errors were seen for ceftazidime, ciprofloxacin and imipenem. No VME was seen for these antibiotics.

Keywords: Conventional microbiology; automation; comparison; nayeem.

1. INTRODUCTION

Clinical microbiology laboratory performs identification (ID) and antimicrobial susceptibility testing (AST) to guide antibiotic therapy and possible drug resistance. Rapid bacterial identification and susceptibility testing improve patient therapy and outcome, decreases emergence of resistance [1,2]. There is a need to provide rapid, efficient and accurate system for identification and antimicrobial susceptibility testing of pathogens. In this regard the automated identification/AST systems aid in rapid diagnosis/treatment of bacterial pathogens [3]. Automated blood culture systems and automated identification and susceptibility testing of bacteria have been in the market for a number of years however application of automated systems in Microbiology is different than other clinical laboratories [4].

Automated systems use sophisticated software to analyze the growth rates and determine the antibiotic minimum inhibitory concentration (MIC) for the organism by using specialized decision technology. Although there are differences among each system the general process of identification is almost same. The Vitek-2 system is the second generation of Vitek and offers a more sophisticated model of data analysis as well as a fully automated process for card identification, organism suspension dilution, and card filling [5].

2. MATERIALS AND METHODS

This was a prospective study conducted in the Department of Microbiology at Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar, Kashmir, a 700 bedded tertiary care hospital. This study was carried for eight months.

Bar coded, inoculated bottles were loaded into the BacT/Alert Microbial Detection System. Blood culture bottles that flagged positive were taken out from the system and subcultured on Blood agar and MacConkey agar to be incubated at 37℃ overnight.

The inocula prepared were processed on to the Vitek-2 system (with software release 2.01) and by manual methods (ID/AST) for comparison. For gram negative bacteria N280 & N 281 cards were used. Manual identification (based on routine spot tests and standard biochemical reactions) and susceptibility testing by conventional methods (Kirby Bauer disc diffusion and micro-broth dilution method) was done for Gram negative organisms [6,7].

2.1 Statistical Analysis

Descriptive statistics (frequency and percentage) was used for the presentation and comparison of data. Appropriate statistical charts were used to present the data.

The “consistent ID” category implied that bacteria were equally identified at the genus and species level by both the conventional and the Vitek-2 systems. For discordant ID results, the assay was repeated with both systems to reconfirm the findings. Results of ID tests obtained with the conventional system were used as a reference.

Antimicrobial susceptibility results were expressed in terms of measure of accuracy. Results of susceptibility tests were categorized as susceptible (S), intermediate (I) or resistant (R) according to criteria recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines. Interpretative values obtained

by Vitek-2 were compared to those obtained with the conventional methods. The following definitions were adopted:

1) Essential agreement (MIC values of Vitek-2 panel equal to or within 1 dilution of the Manual value)
2) Categorical agreement (Vitek-2 and Manual MIC values agree using the interpretative CLSI criteria)
3) Minor errors (Manual is S or R and Vitek-2 is I; alternatively, manual is I and Vitek-2 is S or R)
4) Major errors (Manual is S and Vitek-2 is R). 5) Very major errors (Manual is R and Vitek-2 is S).

3. RESULTS AND DISCUSSION

A total of 135 non duplicate isolates of Gram negative bacteria recovered from the blood samples of the patients admitted at SKIMS were included in the study.

Patients from whom Gram negative isolates were recovered included 83 (61.5%) males and 52 (38.5%) females.

Patients were in the age group of 60-69 years, (n=36; 26.7%) followed by 50-59(n=30; 22.2%), 40-49(n=17; 12.6%), 30-39(n=15; 11.1%), 10-19 (n=11;8.1%), 20-29(n=10;7.4%), ≥ 70(n=9; 6.7%) and 0-9(n=7; 5.2%).

Most of the Gram negative bacteria identified by Vitek-2 included *Escherichia coli* (n=27; 20%) followed by *Klebsiella pneumonia* (n=24; 17.8%), *Acinetobacter baumannii* (n=19; 14.1%), *Enterobacter cloacae* (n=16 ; 11.8%), *Salmonella typhi* (n=14; 10.4%), *Pseudomonas aeruginosa* (n=11; 8.2%), *Citrobacter freundii* (n=8; 5.9%), *Klebsiella oxytoca* (n=7; 5.2%), *Morganella morganii* (n=5; 3.7%), *Acinetobacter lwofii* (n=4; 3%) [Table 1].

Concordant identification (ID) results of Vitek-2 when compared to the manual methods were seen with all the isolates of *Klebsiella pneumoniae, Acinetobacter baumannii, Enterobacter cloacae, Pseudomonas aeruginosa* and *Salmonella typhi.* However discrepancy in results of Vitek-2/manual were seen for 6 isolates of *E. coli* which were *S. typhi* and were misidentified as *E. coli* by the instrument. Likewise 3 isolates of *Klebsiella pneumoniae* were misidentified by Vitek-2 as *Klebsiella oxytoca*. A single isolate of *Citrobacter braakii* was falsely reported as *Citrobacter freundii*. In addition 2 isolates of *Acinetobacter baumannii* were misidentified as *Acinetobacter lwofii* by the Vitek-2 instrument [Table 2].

Table 1. Gram negative bacteria identified by vitek-2

Organism	n	%
Escherichia coli	27	20
Klebsiella pneumonia	24	17.8
Acinetobacter baumannii	19	14.1
Enterobacter cloacae	16	11.8
Salmonella typhi	14	10.4
Pseudomonas aeruginosa	11	8.2
Citrobacter freundii	8	5.9
Klebsiella oxytoca	7	5.2
Morganella morganii	5	3.7
Acinetobacter lwofii	4	3
Total	135	100

Table 2. Identification results for gram negative bacteria

Organism	No of isolates tested	No. (%) of ID's	
		Concordant	Discordant
E. coli	27	21 (77.8%)	6 (22.2%)
K. pneumonia	24	24 (100%)	0
A. baumannii	19	19 (100%)	0
E. cloacae	16	16 (100%)	0
S. typhi	14	14 (100%)	0
P. aeruginosa	11	11 (100%)	0
C. freundii	8	7 (87.5%)	1(12.5%)
K. oxytoca	7	4 (57.1%)	3 (42.9%)
M. morganii	5	5 (100%)	0
A. lwofii	4	2 (50%)	2 (50%)
Total	135	123 (91.1%)	12 (8.9%)

Antibiotic susceptibility profile of Gram negative organisms for all drug classes as given by Vitek-2 and manual method were compared [Table 3]. No minor error (mE), major error (ME) or very major error (VME); with 100% categorical agreement (CA) was seen with ampicillin+sulbactam, piperacillin+tazobactam, ceftriaxone, cefepime, gentamicin, amikacin, levofloxacin, meropenem, colistin, co-trimoxazole, tetracycline, carbenicillin and tobramycin for various Gram negative organisms tested [Tables 4, 5, 6].

However with *E. coli* a mE of 7.4% and a CA of 92.6% was seen for ampicillin and a ME and CA of 3.7% and 96.3% respectively was seen for imipenem. Likewise with *K. pneumonia* a ME rate of 8.3% and a CA rate of 91.7% was seen for ciprofloxacin whereas a ME and CA of 4.2% and 95.8% respectively was seen for imipenem. Also with *P. aeruginosa*, ME rates for ceftazidime, ciprofloxacin and imipenem were 27.3, 9.0, and 18.2% respectively with CA rates of 72.7, 90.9, and 81.8% respectively. No VME was seen for these antibiotics [Tables 4, 5, 6].

Table 3. AST results of gram negative organisms

Gram negative bacteria							
Antibiotic	**Total**	**Vitek-2 n (%)**			**Manual AST n (%)**		
		S	**R**	**I**	**S**	**R**	**I**
Ampicillin	93	2 (2.2)	89 (95.7)	2 (2.2)	2 (2.2)	91 (97.8)	0
Ampicillin/Sulbactam	79	32 (40.5)	47 (59.5)	0	32 (40.5)	47 (59.5)	0
Piperacillin/Tazobactam	121	59 (48.8)	62 (51.2)	0	59 (48.8)	62 (51.2)	0
Ceftazidime	121	39 (32.2)	82 (67.8)	0	42 (34.7)	79 (65.3)	0
Ceftriaxone	124	28 (22.6)	96 (77.4)	0	28 (22.6)	96 (77.4)	0
Cefipime	121	48 (39.7)	73 (60.3)	0	48 (39.7)	73 (60.3)	0
Gentamicin	121	50 (41.3)	71 (58.7)	0	50 (41.3)	71 (58.7)	0
Amikacin	121	47 (38.8)	74 (61.2)	0	47 (38.8)	74 (61.2)	0
Ciprofloxacin	135	14 (10.4)	121 (89.6)	0	17 (12.6)	118 (87.4)	0
Levofloxacin	135	67 (49.6)	68 (50.3)	0	67 (49.6)	68 (50.3)	0
Meropenem	121	53 (43.8)	68 (56.2)	0	53 (43.8)	68 (56.2)	0
Imipenem	121	50 (41.3)	67 (55.4)	4 (3.3)	54 (44.6)	67 (55.4)	0
Colistin	121	121 (100)	0	0	121 (100)	0	0
Co-trimoxazole	124	52 (41.9)	72 (58.1)	0	52 (41.9)	72 (58.1)	0
Tetracycline	110	54 (49.1)	56 (50.9)	0	54 (49.1)	56 (50.9)	0
Carbenicillin	11	4 (36.4)	7 (63.6)	0	4 (36.4)	7 (63.6)	0
Tobramycin	11	5 (45.5)	6 (54.5)	0	5 (45.5)	6 (54.5)	0

Table 4. Comparison of susceptibility test results of *Klebsiella* spp., *E. coli* and *Enterobacter* spp. towards antibiotics between Vitek-2 and conventional method

% Susceptibility									
Antimicrobial	***Klebsiellaspp* (n=31)**			***E. coli* (n=27)**			***Enterobacter spp*(n=16)**		
	CA	**mE**	**ME**	**CA**	**mE**	**ME**	**CA**	**mE**	**ME**
Ampicillin	-	-	-	92.6	7.4	0	100	0	0
Ampicillin/ Sulbactam	-	-	-	100	0	0	100	0	0
Piperacillin/ Tazobactam	100	0	0	100	0	0	100	0	0
Ceftazidime	100	0	0	100	0	0	100	0	0
Ceftriaxone	100	0	0	100	0	0	100	0	0
Cefipime	100	0	0	100	0	0	100	0	0
Gentamicin	100	0	0	100	0	0	100	0	0
Amikacin	100	0	0	100	0	0	100	0	0
Ciprofloxacin	91.7	0	8.3	100	0	0	100	0	0
Levofloxacin	100	0	0	100	0	0	100	0	0
Meropenem	100	0	0	100	0	0	100	0	0
Imipenem	95.8	0	4.2	96.3	0	3.7	100	0	0
Colistin	100	0	0	100	0	0	100	0	0
Co-trimoxazole	100	0	0	100	0	0	100	0	0
Tetracycline	100	0	0	100	0	0	100	0	0

ME: Major error, mE: Minor error, CA: Catagorical agreement

Table 5. Comparison of susceptibility test results of *Citrobacter* spp., *S. typhi* and *Morganella* spp. towards antibiotics between vitek-2 and conventional method

Antimicrobial	% Susceptibility								
	Citrobacter spp (n=8)			*S. typhi* (n=14)			*Morganella spp* (n=5)		
	CA	mE	ME	CA	mE	ME	CA	mE	ME
Ampicillin	100	0	0	100	0	0	100	0	0
Ampicillin/ Sulbactam	100	0	0	-	-	-	100	0	0
Piperacillin /Tazobactam	100	0	0	-	-	-	100	0	0
Ceftazidime	100	0	0	-	-	-	100	0	0
Ceftriaxone	100	0	0	100	0	0	100	0	0
Cefipime	100	0	0	-	-	-	100	0	0
Gentamicin	100	0	0	-	-	-	100	0	0
Amikacin	100	0	0	-	-	-	100	0	0
Ciprofloxacin	100	0	0	100	0	0	100	0	0
Levofloxacin	100	0	0	-	-	-	100	0	0
Meropenem	100	0	0	-	-	-	100	0	0
Imipenem	100	0	0	-	-	-	100	0	0
Colistin	100	0	0	-	-	-	100	0	0
Co-trimoxazole	100	0	0	100	0	0	100	0	0
Tetracycline	100	0	0	-	-	-	100	0	0

ME: Major error, mE: Minor error, CA: Catagorical agreement

Table 6. Comparison of susceptibility test results of *Acinetobacter* spp. and *Pseudomonas* spp. towards antibiotics between vitek-2 and conventional method

Antimicrobial	% Susceptibility					
	Acinetobacter spp (n=23)			*Pseudomonas spp* (n=11)		
	CA	mE	ME	CA	mE	ME
Ampicillin	100	0	0	-	-	-
Ampicillin/ Sulbactam	100	0	0	-	-	-
Piperacillin/ Tazobactam	100	0	0	100	0	0
Ceftazidime	100	0	0	72.7	0	27.3
Ceftriaxone	100	0	0	-	-	-
Cefipime	100	0	0	100	0	0
Gentamicin	100	0	0	100	0	0
Amikacin	100	0	0	100	0	0
Ciprofloxacin	100	0	0	90.9	0	9.0
Levofloxacin	100	0	0	100	0	0
Meropenem	100	0	0	100	0	0
Imipenem	100	0	0	81.8	0	18.2
Colistin	100	0	0	100	0	0
Co-trimoxazole	100	0	0	-	-	-
Tetracycline	100	0	0	-	-	-
Carbenicillin	-	-	-	100	0	0
Tobramycin	-	-	-	100	0	0

ME: Major error, mE: Minor error, CA: Catagorical agreement

3.1 Discussion

In a life threatening infection the important problems are rapid and accurate detection of the pathogen involved. Rapid bacterial identification and susceptibility testing improve patient therapy and outcome, decreases emergence of resistance and also reduces costs [8].

We took 135 isolates of Gram negative bacteria in our study. Donay JL et al. [9] in a comparative study evaluated the identification and antimicrobial susceptibility testing performances of the BD Phoenix Automated Microbiology System took a total of 305 clinical isolates, out of these 187 were Gram negative and 118 were Gram positive, and compared them with manual methods.

In our study Gram negative isolates were recovered from 83 (61.5%) males and 52 (38.5%) females. Similar demographic distribution was observed by Nadheema et al, they found that the frequency of Gram negative bacteria was higher in male than female patients. [10].

In this study the Gram negative bacteria identified by Vitek-2 included *Escherichia coli* (n=27; 20%), followed by *Klebsiella pneumoniae* (n=24; 17.8%), *Acinetobacter baumannii* (n=19; 14.1%), *Enterobacter cloacae* (n=16; 11.8%), *Salmonella typhi* (n=14; 10.4%), *Pseudomonas aeruginosa* (n=11; 8.2%), *Citrobacter freundii* (n=8; 5.9%), *Klebsiella oxytoca* (n=7; 5.2%), *Morganella morganii* (n=5 3.7%), *Acinetobacter lwofii* (n=4; 3%) [Table 1].

A similar type of study was conducted by de Cueto M, et al. [11] and of the 50 Gram negative rods studied, 41 (82%) corresponded to the family Enterobacteriaceae:-24 *Escherichia coli*, 4 *Salmonella spp*., 3 *Klebsiella pneumoniae*, 3 *Proteus mirabilis*, 2 *Klebsiella oxytoca*, and one each *Enterobacter aerogenes, Enterobacter cloacae, Morganella morganii, Citrobacter freundii,* and *Klebsiella ornythinolytica*.

In our study concordant identification in Gram negative organism's results of Vitek-2 when compared to the manual methods were seen with the isolates of *Klebsiella pneumoniae, Acinetobacter lwofii, Citrobacter freundii, Enterobacter cloacae, Pseudomonas aeruginosa* and *Salmonella typhi.*

In our study we got an overall concordance of 91.1% in Gram Negative Bacteria. A study done by Chen JR, et al. [12] in 2008 comparing the Vitek-2 system for positive blood cultures also concluded that Vitek-2 cards provide acceptable identification results for Gram-negative bacilli.

Six (6) discrepant results were seen in case of *E. coli* as reported by Vitek-2 but were identified as *S. typhi* manually. Similar results were seen by Doney et al. while evaluating the automated systems for potential use in Clinical Microbiology Laboratory. The discordance observed in identification results of *S. typhi* in our study may be attributed to the non-availability of biochemical tests for Indole in Gram Negative Cards of the Vitek-2 system. Therefore, they need to be confirmed by -manual methods and by serological testing as recommended by the Vitek-2 manual itself [9,13]. Same could be the reasons for discordance in results as observed in 3 isolates of *K. oxytoca* reported as *k. pneumoniae by* Vitek- 2 and for single isolate of *Citrobacter braakii* reported as *Citrobacter freunddi* by Vitek-2 [14]. Similar discordance was seen by Schreckenberger PC, et al. [15] while Comparing Vitek-2 Colorimetric and Phoenix Systems for Identification of Fermenting and Non-Fermenting Bacteria wherein they found Vitek-2 misidentifying *Citobacter braakii* as *Citrobacter freunddi*.

As for discordant results of 2 isolates of non-fermenters *Acinetobacter baumanii* which were identified incorrectly as *Acinetobacter lwofii* by Vitek-2 in our study, similar results were reported by other investigators and they proposed that this misidentification may be due to the unambiguous separation of Non-fermenting Gram negative bacilli requiring a complex battery of phenotypic test not often present in identification panels, in addition non-fermenting Gram negative bacilli represent several species for which a slow growth is observed; this also represents a handicap for rapid identification methods. [9,16,17,18].

These results are in accordance to another study by Kamm W, et al. [19] where in 204 episodes of septicemia were evaluated. 177 (86.6%) strains were correctly identified with high confidence and 25 (12.5%) gave low-confidence identification. Of these 25 low-confidence identifications, 22 (88%) were correct first-choice identifications and 3 (12%) were incorrect first-choice identifications (1 *E. coli*, 1 *Klebsiella oxytoca*, and 1 *Enterobacter cloacae*). Misidentifications occurred in only 2 episodes (1%), involving 1 *E. coli* and 1 *P. mirabilis*.

Antibiotic susceptibility profile of Gram negative organisms in our study for all drug classes as given by Vitek-2 and reference method are shown in Table 3. No minor error (mE), major error (ME) or very major error (VME); with 100% categorical agreement (CA) was seen with ampicillin+sulbactam, piperacillin+tazobactam, ceftriaxone, cefipime, gentamicin, amikacin, levofloxacin, meropenem, colistin, co-trimoxazole, tetracycline, carbenicillin and tobramycin for various Gram negative organisms tested [Tables 4, 5, 6].

However with *E. coli* a mE of 7.4% and a CA of 92.6% was seen for ampicillin and a ME and CA of 3.7% and 96.3% respectively was seen for imipenem. Likewise with *K. pneumoniae*, a ME rate of 8.3% and a CA rate of 91.7% was seen

for ciprofloxacin whereas a ME and CA of 4.2% and 95.8% respectively was seen for imipenem. Also with *P. aeruginosa*, ME rates for ceftazidime, ciprofloxacin and imipenem were 27.3, 9.0, and 18.2% respectively with CA rates for the same antibiotics of 72.7, 90.9, and 81.8% respectively. No VME was seen for these antibiotics [Tables 4, 5, 6].

Thomas L, et al. while examining 118 isolates found only 97 isolates that had acceptable, good, very good, or excellent identification and were evaluated for the direct susceptibility testing. There was a high percentage of MIC agreement between the Vitek-2 system and the MB method, ranging from 88.7 to 100% for the 11 antibiotics. The Vitek-2 system reported 1,041 (97.6%) correct organism-antibiotic combinations of 1,067 combinations within ±twofold dilution compared with the MB method. The discrepancy rates for ciprofloxacin, piperacillin, and piperacillin-tazobactam were slightly higher than those for other drugs [2].

In another study by N. Stone, et al. [20] out of 12 drugs tested by BMD and DD, 10 showed >95% categorical agreement (CA). CA was lower for ampicillin (80.3%) and cefazolin (77%). There were three very major errors (all with cefazolin, one resolved on repeat testing), one major error (also with cefazolin), and 22 minor errors. Thirty-four of 40 isolates (covering 12 species) that were in the Vitek-2 database were identified correctly to species level, 1 was correct to genus level, and five were reported as unidentified. Vitek-2 generated MIC results for 41 (67.2%) of 61 isolates but categorical interpretations (S, I, R) were provided only for 24. For the 17 drugs tested by both BMD and Vitek-2, essential agreement (41 isolates) ranged from 80.5–100% and CA (24 isolates) ranged from 66.7% (ampicillin) to 100%; twelve drugs exhibited 100% CA.

With regard to AST results, it has been recommended that an overall category error rate of <10% should be obtained for accepting the performance of susceptibility tests, with up to 3.0% major errors and up to 1.5% very major errors (CLSI, Guidelines). With regard to the above guidelines, 100% CA for AST was seen in case of *Enterobacter, Citrobacter, S. typhi, Morgenella, Acinetobacter* species for all antibiotic panels examined in the study. However ampicillin showed a minor error of 7.4% and imipenem showed a major error of 3.7% in case *E. coli*. For *Klebsiella* ciprofloxacin and imipenem showed a major error of 8.3% and 4.2% respectively. For *Pseudomonas* ceftazidime, ciprofloxacin, imipenem showed a major error of 27.3%, 9% and 18.2% respectively.

4. CONCLUSION

The identification part of vitek-2 system has flaws which need to be worked upon especially for the organisms which cause serious life threatening infections (*Salmonella, E. coli*). The treatment modality of the patients changes if the identification of the organism is compromised because separate group of antibiotics need to be employed for treatment. The organisms having slow metabolic rates are prone to errors by the Vitek-2 system. Incorporation of additional biochemical tests like Indole into the Vitek-2 cards can improve the identification and resolve errors where Indole aids in identification (Salmonella*, E. coli, K. pneumonia, K. oxytoca*).

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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