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Klebsiella and Raoultella biotyping and probability of identification by Vitek-2 system

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Abstract: In the present study 800 clinical samples were collected and subjected to biotypes studies. Whereas the present study proved candidates isolates revealed many of biotypes belong the same species of *Klebsiella* and *Raoultella*. Furthermore, this study also demonstrated that isolates which had been candidates to Vitek-2 system,revealed different probabilities,the probability of identification were as follow , excellent 54.7%, very good 7.5%, good 24.5%, acceptable13.2% and low discrimination 1.88% and sum of (excellent ,very good and good) was 86.7%. Also there was high degree of coalition to the level of species and subspecies. In this study we suggested that Vitek 2 system is the best methods to detect biotyping of pathogenic microorganism before studying phylogenetic analysis of microorganisms.

Keywords: Biotypes, *Klebsiella, Raoultella*, Vitek-2, Microbiology, Diagnosis.

I. INTRODUCTION

Klebsiella is a genus of non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule[1] .The genus*Raoultella* is composed of Gram-negative, oxidase-negative, aerobic, nonmotile, capsulated, facultatively anaerobic rods (formerly designated *Klebsiella*) in the family Enterobacteriaceae. It is named after the French bacteriologist Didier Raoult [2].

Automation in clinical microbiology started much later than other clinical laboratories[3]. Furthermore, application of automated systems in clinical microbiology is different than other clinical laboratories. The main difference is the sterile working condition in microbiology and impure clinical bacterial samples which makes one day extra operation for isolation of pure isolated samples. Automated analyze machines still are not popular in daily operation in microbiology laboratories. Where other clinical laboratories are completely replaced automated systems instead of manual methods.Sterile working condition nowadays has been replaced by higher concentrations of bacteria in feeding samples. But pure colonies preparation which is needed for identification process in microbiology still is a problem that needs conventional overnight culture plates. Thus diagnosis process couldnot be start directly after sampling[4].

The diagnosis process in the clinical laboratories starts with a chemical or substrate which exists in sufficient amount before sampling. But in clinical microbiology isolation and reproduction of recordable amount of the pure bacteria are needed before analyze process. Considering these culture and subculture process diagnosis process takes about 2 days. Till now technologic development wouldnot be able to make this preparation interval shorter. But after preparation of pure bacterial colonies auto analyzer machine could be used to reach a shorter diagnosis time. The BioMerieux® introduced Vitek2, the new generation of Vitek® microbiology analyzers and its associated ID-GN card in 1997. The Vitek2 system is developed on fluorescence-based technology and designed for the identification of wide range of micro organisms including gram-negative & gram positive bacteria, Neisseriacea and yeasts in clinical or industrial samples. There are different marked cards containing 64 chambers for identification tests or antibiotic susceptibility testing (AST).In clinical microbiology Vitek®2 used as an auto analyzer system for the identification (ID) and antibiotic susceptibility testing (AST) of the bacteria in clinical samples [5].

II. MATERIALS & METHODS

Samples Collection

A total of 800 clinical samples were collected during study interval(February till September, 2012). These samples were collected from patients attending to Al-Sadder hospital, Al-Hakeem General hospital, Al-Zahraa hospital and Al-Forat Al-Awsat hospitalatAN Najaf, Iraq . All samples were cultured on the MacConkey agar plates and incubated at

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37˚C under aerobic condition for 18 - 24 hour. 250 of lactose ferment which in turn succumbed to extra test to confirm lactose ferment , non motile bacteria represented by *Klebsiella* and Raoultella.

Preservation of bacterial isolates

A. **Short Time Preservation of Bacterial Isolates**

The bacterial isolates were inoculated on nutrient agar slant and preserved at 4˚C. During study, the isolates were maintained monthly by subculture on new nutrient agar medium[6].

B. Long – Term Preservation

Bacterial isolates were maintained for years in brain heart infusion broth as prepared in paragraph (2-1-3-1) containing 15 – 20 % glycerol .This was done by distributing each 200 µl of glycerol in screw – capped tubes and sterilized them at 121 ºC for 10 minutes . Then 800 µl of broth culture was added to each tube and sealed well in parafilm and kept at – 20 ºC.

Inoculum Preparation

Suspension was prepared according to the manufacturer's recommendations of bioMérieux company by transferring sufficient number of colonies from overnight pure culture by swab and suspending the microorganism in 3.0 ml of sterile saline in a 12 x 75 mm clear plastic (polystyrene)(can tube) test tube The turbidity was adjusted to become equivalent to a McFarland No. 5 which had colony forming unit equal to 1.5×10^8. In case of Vitek 2 system the turbidity had been adjusted to be from 0.5 to 0.63 using a turbidity meter called DensiChek. The same suspension was used in identification and antibiogram testing with VITEK-2 compact system.

GN-ID with VITEK-2 Compact System

The identified isolates were confirmed with the automated VITEK-2 compact system by using GN-ID cards. This system identifies an organism via a methodology based on the characteristics of the data and knowledge about the organism and reaction being analyzed. We followed instruction's manufacture in operation VITEK-2 compact system.

STATISTICAL ANALYSIS

The Statistical Package of Science (SPSS) under Microsoft of Windows 7 professional was usedfor data analysis and descriptive statistics analysis (mean, median, and standard deviation) were calculated.

III. RESULTS

Many biotypes had been emerged belong to the same species the **table (1)** show biotypes and bio-numbers for each isolates , the (16) numbers of each biotypes represent the number of biochemical tests of ID-GN36 of VITEK2 system divided by three.

TABLE(1)

Identification and biotyping of clinical isolates by Vitek-2 system.

Isolate number	Vitek identification	bionumber
1	Klebsiella pneumoniae ssp pneumoniae	6607734753564010
2	Klebsiella pneumoniae ssp pneumoniae	6627735753565150
3	Klebsiella pneumoniae ssp pneumoniae	2605734753564610
4	Raoultella ornithinolytica	6637735753577611
5	Klebsiella pneumoniae ssp pneumoniae	6607735753567110
6	Raoultella ornithinolytica	6637734753566411
7	Raoultella planticola	6627735777575052
8	Klebsiella pneumoniae ssp pneumoniae	6607734673564010

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9	Klebsiella pneumoniae ssp pneumoniae	6617735753467031
10	Klebsiella pneumoniae ssp pneumoniae	6617735753567071
11	Raoultella ornithinolytica	6637734753466011
12	Klebsiella pneumoniae ssp pneumoniae	2607735573567650
13	Raoultella ornithinolytica	663773753566011
14	Raoultella ornithinolytica	6637735753577352
15	Klebsiella pneumoniae ssp pneumoniae	6617735751566050
16	Klebsiella pneumoniae ssp pneumoniae	6617735753567031
17	Klebsiella pneumoniae ssp pneumoniae	6627735753565352
18	Klebsiella pneumoniae ssp pneumoniae	6607734773564010
19	Klebsiella pneumoniae ssp pneumoniae	6617714653564010
20	Klebsiella pneumoniae ssp pneumoniae	6617714653564010
21	Klebsiella pneumoniae ssp pneumoniae	6615734653064200
22	Klebsiella pneumoniae ssp pneumoniae	6607735773567411
23	Klebsiella pneumoniae ssp pneumoniae	6607735773567411
24	Raoultella ornithinolytica	6637734753566011
25	Raoultella ornithinolytica	0437530753576211
26	Raoultella ornithinolytica	2637734753066211
27	Klebsiella pneumoniae ssp pneumoniae	6627735753565010
28	Klebsiella pneumoniae ssp pneumoniae	6637735773567272
29	Klebsiella pneumoniae ssp pneumoniae	6607734653564010
30	Klebsiella pneumoniae ssp pneumoniae	6617735773564353
31	Klebsiella pneumoniae ssp pneumoniae	6607734753564010
32	Klebsiella pneumoniae ssp pneumoniae	6607734673064210

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33	Klebsiella pneumoniae ssp pneumoniae	6607734653564410
34	Klebsiella pneumoniae ssp pneumoniae	6637735773565410
35	Klebsiella pneumoniae ssp pneumoniae	6607734753565010
36	Klebsiella pneumoniae ssp pneumoniae	6607734652064010
37	Klebsiella pneumoniae ssp pneumoniae	6627734673164210
38	Klebsiella pneumoniae ssp pneumoniae	6607734653564010
39	Klebsiella pneumoniae ssp pneumoniae	6607734473564210
40	Klebsiella pneumoniae ssp pneumoniae	6607734673164010
41	Klebsiella pneumoniae ssp pneumoniae	6607734653164210
42	Klebsiella pneumoniae ssp ozeanae	4403120240064000
43	Klebsiella pneumoniae ssp pneumoniae	6607734653164010
44	Klebsiella pneumoniae ssp pneumoniae	6607734653564010
45	Klebsiella pneumoniae ssp pneumoniae	6607734653564010
46	Klebsiella pneumoniae ssp pneumoniae	6607734753564010
47	Klebsiella pneumoniae ssp pneumoniae	7627737773564010
48	Klebsiella pneumoniae ssp pneumoniae	7627737673564010
49	Klebsiella pneumoniae ssp pneumoniae	6607734751564000
50	Klebsiella pneumoniae ssp pneumoniae	6607734773564010
51	Klebsiella pneumoniae ssp pneumoniae	6607734773565010
52	Klebsiella pneumoniae ssp pneumoniae	6607734673564010
53	Raoultella ornithinolytica	6735735757576030

The **table(2)** refer to the probability of identification of Vitek2 system or by the other meaning the accuracy of the vitek 2 system, the high property of identification was the excellent probability with % of identification 54.7%,followed by very good , good , acceptable, and low discrimination,which were 7.5%,24.5% and 13.2%

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and 1.88% respectively. The degree of accuracy always determined by the sum of excellent ,very good and good % of identification, which mean in the case of table below equal to 87.4%.

TABLE (2)

Probability of identification of vitek-2

Probability of identification	% of identification
Excellent	54.7
Very good	7.5
good	24.5
acceptable	13.2
Low discrimination	1.88

Probability of excellent identification = 94-99%

Probability of very good identification = 94-95%

Probability of good identification= 89-91%

Probability of low discrimination with extra tests= 91%

Probability of acceptable identification =85-89%

IV. DISCUSSION

The results of this present study demonstrated that isolates which had been candidate to Vitek-2 system,revealed different probabilities,the probability of identification were as follow , excellent 54.7%, very good 7.5%, good 24.5%, acceptable13.2% and low discrimination 1.88% and sum of (excellent,very good and good) was 86.7%. Also there was high degree of coalition to the level of species and subspecies (table 1)&(table 2).As concerning with accuracy of identification, total acceptable and higher results from 80%(sum of acceptable, Good, Very good and Excellent categories) in API system raises till 90% (sum of Excellent, Very good and Good categories) in vitek2 results for 90 clinical samples.Very good 23% Excellent 65% Low Discrimination 5% Good 2% Unidentified organism 5% [5]. Furthermore , as in [7] were investigated total of 118 strains of gram negative bacilli from positive blood cultures which 82.2% strains were correctly identified to the species level (complete coalition), and 17.8% strains were not identified; by comparing the results with those of the reference method of API identification systems. No strain had been misidentified.

. In the same collection of bacteria vitek2 showed 96.5% accuracy and supplemental testing was required to identify only 1% of the results. Of the*Enterobacteriaceae* API 20 correctly identified 90.3% and vitek2 identified 92.4% without supplemental testing. Identification tests with the VITEK 2 system and an API identification system reported 95% of strains was correctly identified to the species level (complete coalition), 2.1% strains were misidentified and 2.8% strains were not identified[8] . Investigation of strains of gram negative rods from patients with cystic fibrosis. The Vitek 2 NGNC identified 94.1% of the non-fermenters correctly. 2.25% were misidentified and 3.6% were determined with low discrimination [9]. In the present study 800 clinical samples were collected and subjected to Biotypes studies. This study also demonstrated that isolates which had been candidated to Vitek-2 system, revealed different probabilities, the probability of identification

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