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RAPID IDENTIFICATION OF *ENTEROBACTER* SPP. ISLATED FROM HOSPITALS IN BASRAH PROVINCE BY AUTOMATED SYSTEM (VITEK®2 COMPACT)

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ABSTRACT: *Atotal of 676 samples were taken from various hospitals in Basrah province. These included clinical specimens(urine , blood , stool ,nasal swabs, throat swabs,ear swabs),Environmental swabs(beds,tables,ground)and milk powder of children.All isolates were subjected to the cultural,microscopical,biochemical examination and vitek2 compact used for identification of bacteria.Atotal of 153 bacterial isolates were diagnosed as Enterobacter(67 isolates E.aerogenes,65 isolates E.cloacae complex,11 isolates E.cloacae subsp cloacae ,4 isolates E.cloacae subsp dissolvens,4 isolates E.sakazakii,1 isolate E.hormaechei and 1 isolate E.asburiae)* .

KEYWORDS: Enterobacter, Vitek®2 Compact, Basrah Province

INTRODUCTION

Enterobacter belongs to domain bacteria, phylum proteobacteria, class gamma-prteobacteria, order enterobacteriales family enterobacteriaceae (Brenner *etal*., 2004). *Enterobacter* was first described by Hormaeche and Edwards(1960*). Enterobacter* are rod-shaped cells,motile by peritrichous flagella,some of which are encapsulated.All *Enterobacter* spp. facultative anaerobes and do not form spores(Mezzatesta *etal.*, 2012).They are biochemically active and ferment sugars such as glucose,arabinose,maltose,xylose,often with gas production. They are oxidase negative,catalase positive and reduce nitrate to nitrite(Murray *etal*.,2003),Voges-proskauer test is usually positive, *Enterobacter* species are ubiquitious and widely found in nature these microorganisms are saprophytic in the environment and commensal in the enteric flora since they are found in soil and sewage, as well as in the gastrointestinal tract of human (Leclerc *et al*.,2001; Mezzatesta *et al.*,2012). It is diverse bacterial genus consisting of several species like *E. aerogenes* and *E. cloacae* have been reported as important opportunistic pathogens for human, These bacteria have been largely described during several outbreaks of hospital-acquired infectious in Europe and particularly in france (Davin-Regli and pages,2015). *Enterobacter* spp. can create community infections are responsible for approximately half of all nosocomial acquired infections(Huang *etal.*,2001). *Enterobacter* has undergone numerous taxonomical rearrangement.Study of Rezzonico *etal*. (2009)indicated that many strains previously identified as *E. agglomerans* and have been transferred into the genus *Pantoea agglomerans*. *E.aerogenes* is considered a homotypic synonym of *Klebsiella mobilis* because it has the same type strain(Skerman *etal.*,1980). Previously reported that *E. sakazakii* was 53-54% related to two genera *Enterobacter* and *Citrobacter* by DNA-DNA hybridization(Farmer *etal.*,1980).*E.sakazakii* was placed in *Enterobacter* genus because of its closer phenotypic and genotypic relationship to *E. cloacae* than to *C,freundii*.

In year 2007 and 2008,classification of *E. sakazakii* with the creation of new genus, *Cronobacter*, has been proposed based on biotyping and genotyping studies (Iversen *etal.*, 2007; Iversen *etal.*,2008).

The routine identification of bacterial isolates in microbiology laboratory is currently done by analysis of phenotypic features such as growth on selective and nonselective media ,colonial morphology ,Gram-stain,biochemical reactions .These methods are laborious,time-consuming (Darbandi,2010;Jamal.;2014).

The vitek 2 compact is an automated microbiology system utilizing growth based technology and designed for the identification and susceptibility testing of wide range of micro organisms including Gram-negative and Gram positive bacteria and yeasts in clinical or industrial samples(Ling *et al* 2001;Darbandi,2010).This system use colorimetric reagent cards that are incubated and interpreted automatically(Pincus.2005). Figure (1) shows the VITEK 2 compa**ct.** The purpose of this study was to identify of *Enterobacter* spp. isolated from different areas by using automated systemVitek®2 compact.

MATERIALS AND METHODS

Samples collection

Six hundred and sixsty seven samples were collected from different areas of Basrah hospitals (Al-Fayhaa General hospital ,Al-Mawanee General hospital , Al-Sadder teaching hospital , Al-Basrah hospital for gynecology and obstetrics , Al-Basrah children's specialty hospital, Al-Basrah General hospital).The collected samples represent clinical and environmental samples ,Clinical specimen including blood, urine, stool, nasal swabs , throat swabs , ear swabs) .Environmental swabs were taken from beds,tables,ground and food samples represented by milk powder of children'patients suffering from diarrhea.All samples were collected under sterile conditions and sent to the laboratory within 1-2 hrs.

Isolation and Identification of Bacteria.

Isolation from milk powder(FDA,2002)

One gram of milk powder of infants from each sample was mixed with 9ml sterile peptone water.The tubes were then incubated for 24hrs at 37 °C, and 0.1ml of each suspension was streaked on violet red bile agar (VRBA) the plates were incubated for24hrs at 37 °C ,red colonies appeared on VRBA were subcultured by streaking on tryptic soya agar (TSA) and

incubated for 24hrs at 25°C.The colonies that produce yellow pigment were identified using traditional biochemical tests and vitek2 compact.

Isolation from other specimens.(Mano and Byku,2012)

All specimens were inoculated on various ordinary media; blood agar, MacConkey agar, EMB agar and incubated at 37 °C for 24 hrs under aerobic conditions, after that the culture plates were examined according to the appearance, color and morphology of the colonies and Positive cultures were subjected to biochemical tests(sugar fermentation, IMVC, TSI,Oxidase,Catalase) for identification of bacteria.

Confirmatory Identification of *Enterobacter* spp by Vitek® 2 compact

All isolated were cultured on nutrient agar and incubated for 24hrs at 37 °C to ensure purity and to get single colonies ,after isolation of bacterial colonies on culture media,isolates were identified by Vitek® 2 compact auto analyzer system manufactured by (BioMerieux,USA)Public health lab. In Najaf and this process including several steps(Pincus,2005;Darbandi,2010):

1-Asterile plastic stick applicator used to take pure colonies from culture media and transfer sufficient number of them to clear plastic(polystyrene) test tube 12×75 mm contain about 3 ml of sterile saline(NaCl 0.45%-0.50%,pH=7)BioMerieux,USA to suspend the microorganism in.
2-Concentration of bacterial suspension in saline was measured by a densitometer and adjusted to 0.50-0.63 Mcfarland before introducing the sample to the analyzer.
3-The turbidity of bacterial suspension was adjusted by adding proper amount of bacteria or normal saline and mixing by shaker to produce a homogenous suspension of bacteria.
4-The turbidity (the density) of the suspension was checked by using a calibrated turbidity meter called the Densichek
5-Identification GN cards were loaded (inoculated) with bacterial suspension using an integrated vacuum apparatus.
6-A test tube containing the bacterial suspension is placed into a special rack(cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube, the cassette contain place for 10 test tubes.
7-The filled cassette was placed into a vacuum chamber station inside the vitek® 2 compact machine .
8- After the vacuum is applied and air is re-introduced into the station,the bacterial suspension was forced through the transfer tube into micro-channels that filled all the test wells.
9- Inoculated GN cards were passed by a mechanism,which cut off the transfer tube and sealed the card prior to loading into the circular incubator.
10-Circular incubator could accommodate up to 30 cards,all card types were incubated at 35.5+1 °C.
11-Each card was removed from the incubator every 15 minutes,transported to the optical system for reaction readings, and then returned to the incubator until the next read time.
12-Data were collected at 15 minute intervals during the entire incubation period**.**
13- Vitek 2 compact tests listed in Table (1)

Table (1) Test Substrates of Vitek 2 compact

Well number	Mnemonic	Biochemical Test
2	APPA	Ala-Phe-Pro-ARYLAMIDASE
3	ADO	ADONITOL
4	PyrA	L-Pynolydonyl-ARYLAMIDASE
5	IARL	L-ARABITOL
7	dCEL	D-CELLOBIOSE
9	BGAL	BETA-GALACTOSIDASE
10	H_2S	H2SPRODUCTION
11	BNAG	BETA-N-ACETYL-GLUCOSAMINIDASE
12	AGLTo	Glutamyl Arylamidase pNA
13	dGLU	D-GLUCOSE
14	GGT	GAMMA-GLUTAMYL-TRANSFERAS
15	OFF	FERMENTATION/GLUCOSE
17	BGLU	BETA-GLUCOSIDASE
18	dMAL	D-MALTOSE
19	dMAL	D-MANNITOL
20	dMNB	D-MANNOSE
21	BXYL	BETA-XYLOSIDASE
22	BAlap	BETA-Alanine arylamloase pNA
23	ProA	L-Proline ARYLAMIDASE
26	LIP	LIPASE
27	PLE	PALATINOSE
29	TyrA	Tyrosine ARYLAMIDASE
31	URE	UREASE
32	dSOR	D-SORBITOL
33	SAC	SACCHAROSE/SUCROSE
34	dTAG	D-TAGATOSE
35	dTRE	D-TREHALOSE
36	CIT	CITRATE(SODIUM)
37	MNT	MALONATE
39	5KG	5-KETO D-CLUCONATE
40	ILATk	L-LACTATE alkalinisation
41	AGLU	ALPHA-GLUCOSIDASE
42	SUCT	SUCCINATE alkalinisation
43	NAGA	Beta-N-ACETYL-GALACTOSAMINIDASE
44	AGAL	ALPHA-GALACTOSIDASE
45	PHOS	PHOSPHATASE
46	GlyA	Glycine ARYLAMIDASE
47	ODC	ORNITHINE DECARBOXYLASE
48	LDC	LYSINE DECARBOXYLASE
52	ODEC	DECARBOXYLASE BASE
53	IHISa	L-HISTIDINE assimilation
56	CMT	COUMARATE
57	BGUR	BETA-GLUCORONIDASE
58	O129R	O/129RESISTANCE(comp.vibrio)
59	GGAA	Glu-Gly-Arg-ARYLAMIDASE

61	IMLTa	L-MALATE assimilation
62	ELLM	ELLMAN
64	ILATa	L-LACTATE assimilation

RESULTS

A total of 676 Clinical , environmental and food specimens were collected during the period(January 2013-December 2013),from Basrah hospitals,The clinical specimen included blood(50) ,urine(100) ,stool (100), nasal ,throat and ear (50) and environmental specimens included patient bed swabs (200) , tables swabs (100),patient room ground swabs (50) and food specimens (milk powder of infants specimens (26)(Table ,2). 170 *Enterobacter* spp. were identified during this study.

Table (2): Distribution *Enterobacter* spp. in various samples

Sample	No(%)0f samples	Number *Enterobacter* spp.					Total number of *Enterobacter* spp.	
		EC.	***E. aero genes***	***E. saka zakii***	***E. horma echei***	***E. asbu riae***	**N**	**%**
Clinical specimens	7.4%	*EC1=1	0	0	0	0	1	0.7
Blood(n=50)								
Urine(n=100)	14.79%	EC1=1	4	0	0	0	5	3.3
Stool(n=100)	14.79%	EC1=1 **EC2=1	4	0	0	0	6	3.9
Nasal,Throat,ear(n=50)	7.4%	0	0	0	0	0	0	0
Environmental specimens	29.59%	EC1=44 EC2=10 EC3=3	51	2	0	0	110	71.9
Patient bed(n=200)								
Patient tables in different hospitals(n=100)	14.79%	EC1=16 ***EC3=1	7	2	1	0	27	17.6
Patient room ground in different hospitals(n=50)	7.4%	EC1=2	0	0	0	0	2	1.3
Food specimens	3.8%	0	1	0	0	1	2	1.3
Milk powder infant(n=26)								
							Total=153	

*EC1=*Enterobacter cloacae* complex
**EC2=*Enterobacter cloacae* subsp cloacae
***EC3=*Enterobacter cloacae* subsp dissolvens

Identification and characterization of *Enterobacter* spp.

Characterization of *Enterobacter* spp. used in the present study was carried out in accordance with conventional methods(Gram stain,morphological characterization and biochemical tests)(Table,3) . All isolates were also confirmatory identified by using Vitek 2 compact GN colorimetric card was read and interpreted automatically with Vitek 2 compact system.

Table (3): Morphological and Biochemical tests which were used to identify *Enterobacter* spp.

No.	Biochemical test	Result
1	Gram stain	-
2	Morphological shape	Rod
3	Indole test	-
4	Methyl red test	-
5	Voges-Proskauer test	+
6	Citrate utilization test	+
7	TSI,B/S	+/+,G
8	Gelatin hydrolysis	+/-
9	Oxidase test	-
10	Catalase test	+
11	Motility test	+
12	Aesculin hydrolyzed	V
13	Utilization of raffinose	V
14	DNase test	-

(+)=Positive,(-) =Negative,(V) =Variable,G=Gasis produced,B/S=Butt/Slant

TSI=Triple sugar iron

Identification of *Enterobacter* spp. by Vitek 2 compact

Identification of 170 *Enterobacter* species depending on biochemical reactions (Table ,3)were confirmed by Vitek 2 compact .Vitek 2 Results showed that(153) isolates were identified as *E.* species as following 65(42.5%)isolate were identified as *E.cloacae* complex, 11(7.2%)isolate were identified as *E.cloacae* spp cloacae , 4(2.6%) isolate were identified as *E.cloacae* spp dissolvens, 67(43.80)isolate were identified as *E.aerogenes*, 4(2.6%)isolate were identified as *E.sakazakii* , 1(0.7%) isolate was identified as *E.hormaechei*, 1(0.7%) isolate was identified as *E.asburiae* (Fig ,2). The other (17) isolates were unidentified by Vitek 2 compact. Among all bacterial isolates obtained only 12(7.84%) *Enterobacter* spp were isolated from clinical specimens and recognized as (3)*E cloacae* complex,(1)*E. cloacae* ssp cloacae,(8)*E. aerogenes*, while 139(90.85%)isolates of *Enterobacter* spp were isolated from hospital environmental swabs and recognized as(62)*E. cloacae complex*,(10) *E.cloacae* ssp. cloacae,(4)*E.cloacae* ssp. dissolvens (58) *E.aerogenes* (4)*E.sakazakii*,(1)*E.hormeachei* .Also 2(1.31%)of *Enterobacter* spp were isolated from infant milk powder and recognized as (1)*E.aerogenes*,(1)*E.asburiae* Table(4).

The results revealed that the isolates identified at the species level was divided into four groups based upon the probability of accurate identification as follows: 59.48% isolate were excellent(probability of accurate identification(96-99%),28.10% isolate were very good(93t o 95%),11.76% isolate were good(89 to 92%) and 0.70% isolate acceptable(85 to 88%) as gave in Fig(3).

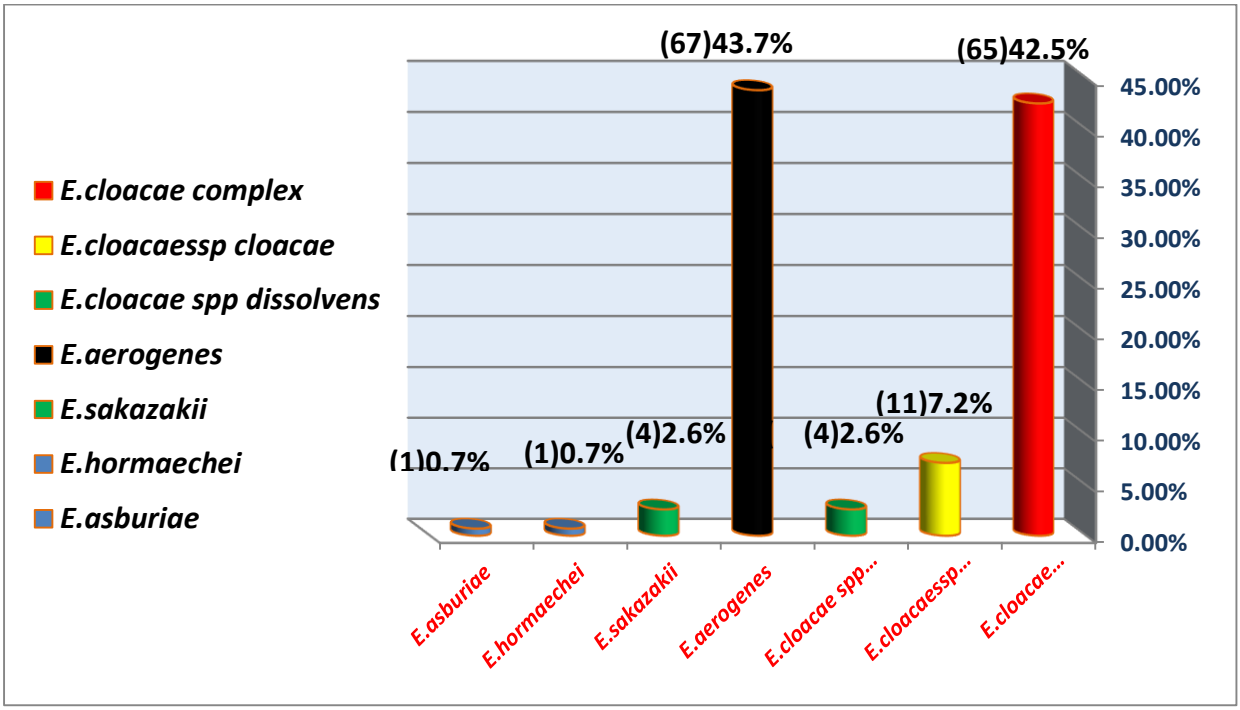
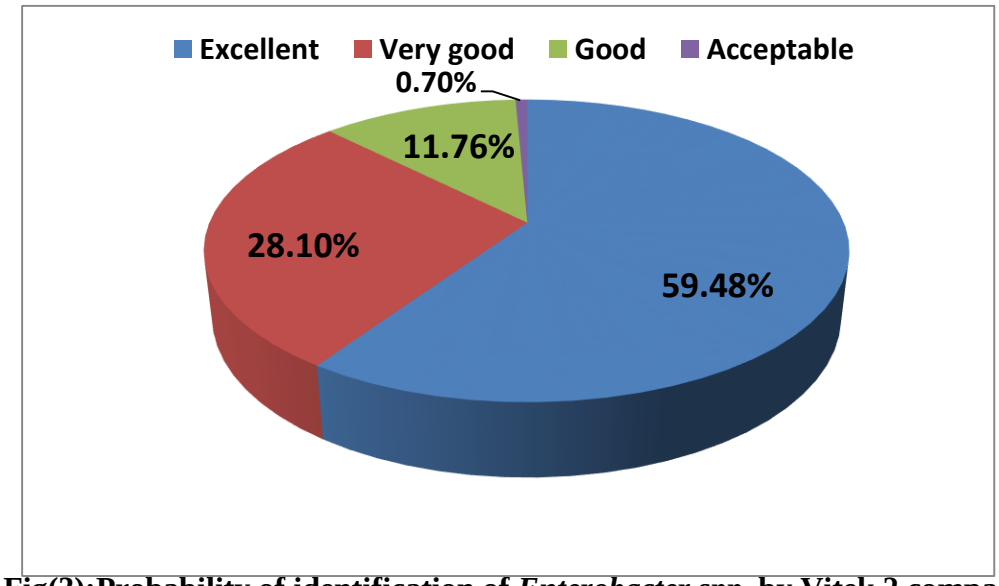


Figure (2)Percentage of *Enterobacter* spp.isolated from different samples by using vitek 2 compact

Table (4): Number of samples collected and *Enterobacter* spp. isolated from clinical , Environmental and food specimens



Fig(3):Probability of identification of *Enterobacter spp.* by Vitek 2 compact

Probability of excellent identification =96-99%
Probability of very good identification =93-95%
Probability of good identification =89-92%
Probability of Acceptable identification =85-88%

DISCUSSION

Infections due to Gram negative bacteria has become an increasing problem in recent years(Sankarankutty and Kaup,2014).The present study revealed among 676 samples from(clinical , food and environment of Basrah hospitals) that153 were *Enterobacter* spp and most bacterial isolates from hospital environments, The results showed that *Enterobacter* spp were distributed as follow:139(90.85%)from hospital environmental specimens,12(7.84%)from clinical specimens,2(1.31%)from milk powder specimens as showed in Table(4),These results indicated wide distribution of *Enterobacter* spp in the environment of hospitals and this agreement with other studies(Jalaluddin *etal*.,1998;NNIS,2004;Chang *etal*.,2009).*Enterobacter* is more a nosocomial opportunistic pathogen that cause variety of hospital acquired infections (Sanders and sanders,1997;Gupta *etal*.,2003). National healthcare safety network reported that *Enterobacter* account for approximately 5% of nosocomial bacteremia in 2008(Hidron *etal*.,2008).especially in intensive care units(ICUs)(Boban *etal*.,2011).Hospital environments are responsible of the dissemination of microorganism for different distances and progressive contamination of surfaces,water and air (Boyce etal.,1997;Curtis,2008).The results showed that a higher percentage(43.80%)of isolates were identified as *E. aerogenes* followed by *E.cloacae* complex(42.5%),*E.cloacae* sub sp cloacae(7.2%),*E.cloacae* sub sp dissolvens *and E.sakazakii* with the same percentage(2.6%),*E.hormaechei* and *E.asburiae* with the same percentage(0.7%).These results indicated the most frequently *E.aerogenes and E.cloacae* complex and less frequently *E.hrmaechei* and *E.asburiae* as reported previously(Yu *et al*.,2000). Al-Tawfig *et al*.(2009) also proved that *E.cloacae* and *E. aerogenes* constituted 60% and 33% of their isolates,respectively .

Hussain and Alammar(2013) recorded higher percent for *E. cloacae* (89.3%)from various hospitals of Najaf/Iraq,on the other hand *E. aerogenes* is considered the fifth highest Enterobacteriaceae and the seventh highest Gram negative Bacilli responsible for notorious nosocomial infections in france(Carbonne *et al*.,2013).*E.aerogenes* and *E.cloacae* have been largely described during several outbreaks of hospital acquired infection in Europe especially in France because these bacterial species are able to acquire numerous genetic mobile elements that contribute to antibiotic resistance,this help them to colonize several environments and host and rapidly adapt their metabolism to external conditions and environmental stresses(Davin-Regli and Pages,2015).

Identification of *Enterobacter* spp

Identification of *E*. spp depended on morphological ,microscopic examination and biochemical tests. revealed that all *Enterobacter* isolates were gram negative and rod shape according to results recorded in Bergey's manual of determinative bacteriology(1994) and Bergey's manual of systematic bacteriology(2004) (Holt *etal*.,1994;Brenner *etal*.,2004).

Identification of *Enterobacter* spp by Vitek 2 compact

Identified isolates of *Enterobacter* by convensional methods were confirmed with the automated vitek2compact system by using GN-ID cards.In this study 153 isolate identified to species and subspecies level of *Enterobacter* this depended on difference in colorimetric

measurements that taken every 15 minutes by vitek 2 compact system for each isolate.Observed during the present results some isolates had the same species and appeared differentce in some biochemical tests this indicated they belong to different strains, strains identification at the species level were divided into four groups as showed in fig(3) based on the probability of accurate identification as follows excellent (probability of accurate identification ≥96%),very good(93-95%),good(89-92%)and acceptable(85-88%)(Zbinden *et al.*,2007).Vitek 2 compact system had several advantages ,it identified a significant number of Gram negative bacteria during 6 hrs which clinically relevant,because rapid reporting of microbiology results compared with traditional methods that require two or three days and has high level of automation ,a simple methodology and taxonomically updated databases (Ling *etal*.,2003;Wallet *et al*.,2005;Otto-Karg *et al*.,2009;Dina *et al.*,2014).Vitek 2 compact incorporates several technical improvements which automate many procedures that performed manually with the previous vitek system(Decueto *et al*.,2004).

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