

Full Length Research Paper

Special biochemical profiles of *Escherichia coli* strains isolated from humans and camels by the VITEK 2 automated system in Al-Ahsa, Saudi Arabia

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The VITEK 2 automated system was used for comparing biochemical characteristics of *Escherichia coli* isolates from human urinary tract infections (UTIs) and camel faecal samples for the first time in the study area. Identification by the system to the species level was accurate. Recovery rate of *E. coli* from camel specimens was 26% and for human specimens was 33%. Based on biochemical activities, human and animal strains were distributed into 19 profiles. Biochemical profiles 1 and 2, of the classical *E. coli* activity, comprised 26 camel (50%) and 16 human strains (24.2%). The rest human strains (75.8%) were distributed among 10 profiles and 50% camel strains among 7 profiles. *E. coli* O157 was not confirmed as 6.1% human isolates were β -glucuronidase negative but sorbitol positive whereas, 11.5% camel isolates were sorbitol negative and β -glucuronidase positive. The results showed atypical biochemical reactions but no unique biochemical profile number for *E. coli* causing community-acquired UTIs in the study area. Phenotypic similarity between camel and human isolates was demonstrated and implication of camel isolates in environmental contamination is discussed.

Key words: *Escherichia coli*, non-O157, VITEK 2, camel, human, Saudi Arabia.

INTRODUCTION

Escherichia coli is a member of Family Enterobacteriaceae that live as a commensal in the intestinal tract of humans and animals but occasionally may cause infection in the intestinal tract and other body systems. *E. coli* is Gram negative, rod-shaped, non-spore forming, motile with peritrichous flagella or non-motile and about 2.0 μm long and 0.25 - 1.0 μm in diameter.

They are able to grow under aerobic and anaerobic conditions. Optimal growth occurs at 35-37°C (Koneman et al., 2005).

Traditionally, biochemical reactions are used for identification and confirmation of bacteria to species level. All Enterobacteriaceae are oxidase negative except *Pleisomonas shigelloides*. *Escherichia* species are

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positive for indole. It ferments dextrose (D-glucose) by producing mixed acids (e.g. lactic, acetic and formic acids) that can then be made visible with the addition of an indicator sensitive to pH change as phenol red or methyl red. *E. coli* is catalase positive, oxidase negative and reduces nitrates. There are many other biochemical tests to indicate the presence of *E. coli*. For instance, Voges and Proskauer found a test to detect acetoin and 2,3-butanediol produced when *Klebsiella* and *Enterobacter* ferment glucose. The researchers found that under alkaline conditions, these two compounds oxidize themselves into diacetyl. Diacetyl then reacts with creatine (a guanidine derivative) and appears as a pinkish-red compound, or it reacts with α -naphthol and appears cherry-red in colour (Koneman et al., 2005).

Kingdom of Saudi Arabia (KSA) has a wealth of camel (*Camelus dromedaries*) population that provide milk, meat, wool, hides and skin. In rural areas, a close relationship occurs between camels and their owners.

Extra-intestinal pathogenic *E. coli* (ExPEC) is a diverse *E. coli* pathogenic type with genetic diversity which is reflective of its colonization of widespread ecological niches (Singer, 2015). Among these, urinary tract infections (UTIs) are one of the most common reasons for attendance at primary and secondary healthcare services. There are an estimated 150 million UTIs every year worldwide (Russo and Johnson, 2003). Enterohemorrhagic strains of *E. coli*, especially *E. coli* O157, have emerged as important enteric pathogens in recent years. The group produces a toxin almost identical to that of *Shigella dysenteriae* and this is responsible for the gastroenteritis in man, which ranges in severity from mild to bloody diarrhea and hemorrhagic colitis. Some patients develop hemolytic uremic syndrome (HUS) with anemia and acute renal failure. Some farm animals are infected with *E. coli* O157 without showing signs of the illness, that is, they are sub-clinically infected. Feces from these animals may contain *E. coli* O157 in varying numbers. *E. coli* O157 is generally identified as being a non-sorbitol fermenting, Gram negative rod shaped organism, ranging from 0.7 to 1.5 \times 2 to 5 μ m in size, oxidase negative, catalase positive and indole positive (ISO, 2001).

Evidence accumulates that *E. coli* populations in the GI tract of the human host changed frequently over time through clonal replacement, but the ecological and genetic reasons for these changes were never clarified (Caugant et al., 1981). Similar observations of a high rate of *E. coli* turnover in the GI tract have also been made in animal populations (Hinton, 1986). Natural selection may increase the frequency of new beneficial mutations as standing genetic variation. It is not yet well understood how different features of population biology or different environmental circumstances affect these adaptive processes (Burke, 2012). Multilocus sequence typing (MLST) provides an efficient genotyping tool for molecular epidemiology analysis. *E. coli* strains with

identical MLST profiles (known as sequence types or STs) may possess distinct genotypes. This enables different ecotypic or pathotypic lifestyles. However, STs are not uniform with regard to genetic properties or ecotypic/pathotypic behaviors (Weissman et al., 2012).

The VITEK 2 Automated System (bioMérieux, Marcy L'Etoile, France) is one of the most widely used systems in clinical microbiology laboratories for the identification of bacteria up to species level. The system uses reagent cards that have 64 wells, each with individual substrate for sugar utilization, enzymatic and biochemical tests. Identification cards are inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension is placed into cassette and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube then the vacuum is applied and air is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells (Pincus, 2006). Basically, it is a colorimetric reading of biochemical reactions of microorganisms. Based on these readings, an identification profile is established and interpreted according to a specific algorithm. Final profile results are compared with the database, generating identification of the unknown organism. Final results are analyzed using a software which is an Advanced Expert System (AES) specifically designed to evaluate the results generated by the VITEK 2 system. Testing is repeated wherever suggested by the AES.

Many bacterial species can be transmitted between animals and humans, either through the food chain, via the environment, or by physical contact. Transmission of extra-intestinal infections by *E. coli* from food animals could be responsible for human infection (Bergeron et al., 2012). Normal bacterial flora in the body of camels may benefit the host; occasionally, may be source of infection. From a public health perspective, camel may act as reservoir for *E. coli* infection.

Studies on the phenotypic characteristics of *E. coli* living as commensals in the gut of dromedaries camel are very few. Hence, the goal of this study was to determine phenotypic similarities of *E. coli* isolates from humans and camel. The hypothesis for this investigation is that *E. coli* recovered from fecal samples of camels is a reservoir for *E. coli* causing community-acquired UTI in the study area.

MATERIALS AND METHODS

Animal specimens

Freshly voided feces (200 samples) were collected from camel farms in Al Ahsa Province in sterile containers and transferred immediately in icebox to the laboratory. To prepare the samples, 1 g of fecal sample was dissolved in 9 mL sterile physiological saline for culturing (Manyi-Loh et al., 2014).

Human specimens

The study population consisted of positive cultures of urine samples (therefore no ethical approval or informed consent was required) from female patients diagnosed with UTIs, who had samples sent to the medical diagnostic laboratory of King Fahad Hospital, Al-Ahsa for culture and sensitivity testing. *E. coli* isolates from a total of 200 urine samples were randomly selected to be included in the study.

Laboratory procedures

Camel specimens were streaked onto blood agar (Oxoid, Basingstoke, UK) and MacConkey agar plates (Oxoid). The plates were incubated at 37°C for 24 h. Culture characteristics and microscopic features were observed and recorded for presumptive identification, as described by Koneman et al. (2005).

Human specimens were sub-cultured on blood and MacConkey agar, incubated at 37°C for 24 h and prepared for identification as described by Koneman et al. (2005). Sorbitol MacConkey agar (SMA) (Oxoid) was used to type *E. coli* O157 from the obtained human and animal isolates.

VITEK 2 GN identification procedure

Confirmation of the identification of isolates was performed using the VITEK 2 technique (Valenza et al., 2007).

A bacterial suspension made in 0.45% aqueous NaCl was adjusted to a McFarland standard of 0.5 with a VITEK 2 DensiCheck instrument (bioMérieux). The card for biochemical tests for Gram negative bacterial species which consists of 47 substrates (Table 1) was used. Result interpretation was done by comparing an unknown biochemical pattern to the database of reactions for each taxon and a numerical probability was calculated. Various levels of identification were assigned based on numerical probability calculations as, excellent (% probability 96-99), very good (% probability 93-95), good (% probability 89-92) and acceptable (% probability 85 to 88).

RESULTS

Animal specimens

From camel fresh feces, a total of 200 specimens were examined. *E. coli* in pure culture was isolated and identified by conventional methods. Cultural characteristics on Blood agar and MacConkey agar were studied together with microscopic examination of microbiological smears stained with Gram's stain. Lactose-fermenting colonies on MacConkey agar were suggestive to be *E. coli*.

Hemolytic activity

Five (9.6%) camel strains showed β -hemolysis on blood agar plates. All the isolates were confirmed by VITEK 2 technique. *E. coli* was confirmed from 52 specimens with recovery rate of 26% by the biochemical tests of VITEK 2 technique (Table 2).

Identification of *E. coli* O157 strains

Testing of *E. coli* isolates on SMA, showed six isolates (11.5%) with clear colorless colonies after incubation for 24 h at 35°C. The isolates were considered to be non-sorbitol fermenting and presumptive *E. coli* O157 strains. The rest of the isolates gave pink colonies indicating sorbitol fermentation.

From 200 human UTI specimens, *E. coli* was isolated and confirmed from 66 cases giving a percentage of 33% by the biochemical tests of Viteck 2 technique (Table 3). On SMA, all human strains were sorbitol fermenters.

Hemolytic activity:

A total of 14 (21.2%) human strains showed β -hemolysis on blood agar plates. Based on biochemical activities, all 118 human and camel *E. coli* strains were divided into 19 biochemical profiles. Profiles were different at least in one of the reactions tested.

Biochemical profile 1(P1), showing classical *E. coli* biochemical activities, was represented by 12 human and 16 camel isolates (Table 2). Profile 2 (P2) was represented by 4 human and 10 camel isolates which differed from P1 strains only by being positive for gamma-glutamyl-transferase. Profiles 3 – 12 (P3 – P12) contain human strains and profiles 13 – 19 (P13 – P19) contain camel strains. P3 was negative for D-mannose while P4 was positive for malonate and both contained 4 human strains. P5 was negative for D-maltose, positive for L-proline arylamidase and P6 positive for sucrose and glycine arylamidase. P7 – P9 gave odd reaction to four biochemical tests. P7 (4 strains), had odd reactions to L-lactate alkalisation, glycine arylamidase, ornithine decarboxylase, O/129 Resistance; P8 (8 strains), odd to L-proline arylamidase, D-tagatose, phosphatase, glycine arylamidase. P9 (4 strains), odd to sucrose, D-tagatose, phosphatase, glycine arylamidase. P10 – P11 gave odd reaction to five biochemical tests. P10 (4 strains), odd to tyrosine-arylamidase, sucrose, L-lactate alkalisation, succinate alkalisation, alpha-galactosidase. P11 (6 strains) was odd to tyrosine-arylamidase, sucrose, L-lactate alkalisation, succinate alkalisation, O/129 Resistance. P12 (4 strains) was odd to gamma-glutamyl-transferase, D-tagatose, 5-keto-D-gluconate, phosphatase, glycine arylamidase, ornithine decarboxylase and beta-glucuronidase. In camel strains, P13 – P15 gave odd reaction to only one biochemical test. P13 (4 strains), was odd to L-proline arylamidase; P14 (2 strains), was odd to sucrose; P15 (2 strains), odd to phosphatase. P16 – P18 gave odd reaction to two biochemical tests. P16 (4 strains) was odd to sucrose and phosphatase; P17 (4 strains) was odd to L-proline arylamidase and sucrose; P18 (4 strains) was odd to D-maltose and phosphatase. P19 (6 strains) was odd to four biochemical tests: gamma-glutamyl-transferase, D-mannose, L-proline arylamidase and D-sorbitol.

Table 1. VITEK 2 biochemical test substrates and amount per well on the card for Gram negative bacterial species.

Well	Abbreviation	Test substrate	Amount/well (mg)
2	APPA	Ala-Phe-Pro-Arylamidase	0.0384
3	ADO	Adonitol	0.1875
4	PYRA	L-Pyrrolydonyl-Arylamidase	0.018
5	IARL	L-Arabitol	0.3
7	DCEL	D-Cellobiose	0.3
9	BGAL	Beta-Galactosidase	0.036
10	H2S	H2S Production	0.0024
11	BNAG	Beta-N-Acetyl-Glucosaminidase	0.0408
12	AGLTP	Glutamyl Arylamidase pNA	0.0324
13	dGLU	D-Glucose	0.3
14	GGT	Gamma-Glutamyl-Transferase	0.0228
15	OFF	Fermentation/Glucose	0.45
17	BGLU	Beta Glucosidase	0.036
18	dMAL	D-Maltose	0.3
19	dMAN	D-Mannitol	0.1875
20	dMNE	D-Mannose	0.3
21	BXYL	Beta-Xylosidase	0.032
22	BALAP	Beta-Alanine- Arylamidase pNA	0.0174
23	PROA	L-Proline Arylamidase	0.0234
26	LIP	Lipase	0.0192
27	PLE	Palatinose	0.3
29	TyRA	Tyrosine-Arylamidase	0.0276
31	URE	Urease	0.15
32	dSOR	D-Sorbitol	0.1875
33	SAC	Saccharose/Sucrose	0.3
34	DTAG	D-Tagatose	0.3
35	dTRE	D-Trehalose	0.3
36	CIT	Citrate (Sodium)	0.054
37	MNT	Malonate	0.15
39	5KG	5-Keto-D-Gluconate	0.3
40	ILATK	L-Lactate alkalisation	0.15
41	AGLU	Alpha-Glucosidase	0.036
42	SUCT	Succinate alkalisation	0.15
43	NAGA	Beta-N-Acetyl-Galactosaminidase	0.0306
44	AGAL	Alpha-Galactosidase	0.036
45	PHOS	Phosphatase	0.0504
46	GLYA	Glycine Arylamidase	0.012
47	ODC	Ornithine Decarboxylase	0.3
48	LDC	Lysine Decarboxylase	0.15
53	IHISA	L-Histidine assimilation	0.087
56	CMT	Coumarate	0.126
57	BGUR	Beta-Gluconidase	0.0378
58	O129R	O/129 Resistance (comp.Vibrio.)	0.0105
59	GGAA	Glu-Gly-Arg-Arylamidase	0.0576
61	IMLTA	L-Malate assimilation	0.042
62	ELLM	Ellman	0.3
64	ILATA	L-Lactate assimilation	0.186

DISCUSSION

In the present study, identification of *E. coli* by the Vitek 2 technique was excellent or very good. Crowley et al. (2012) in an evaluation study of the technique, concluded

that the VITEK 2 GN identification method is an acceptable automated method for the rapid identification of Gram-negative bacteria.

Based on biochemical activities, *E. coli* human strains were divided into 12 biochemical profiles and camel

Table 2. Biochemical profiles of *E. coli* isolates of profiles 1 and 2 from camel faecal samples and human urinary tract infection specimens from Al Ahsa Province, KSA.

Code	Reagent	Profile 1	Profile 2
2	APPA	-	-
3	ADO	-	-
4	PYRA	-	-
5	IARL	-	-
7	DCEL	-	-
9	BGAL	+	+
10	H2S	-	-
11	BNAG	-	-
12	AGLTP	-	-
13	dGLU	+	+
14	GGT	-	+
15	OFF	+	+
17	BGLU	-	-
18	dMAL	+	+
19	dMAN	+	+
20	dMNE	+	+
21	BXYL	-	-
22	BALAP	-	-
23	PROA	-	-
26	LIP	-	-
27	PLE	-	-
29	TyRA	+	+
31	URE	-	-
32	dSOR	+	+
33	SAC	-	-
34	DTAG	-	-
35	dTRE	+	+
36	CIT	-	-
37	MNT	-	-
39	5KG	-	-
40	ILATK	+	+
41	AGLU	-	-
42	SUCT	+	+
43	NAGA	-	-
44	AGAL	+	+
45	PHOS	+	-
46	GLYA	-	-
47	ODC	+	+
48	LDC	+	+
53	IHISA	-	-
56	CMT	+	+
57	BGUR	+	+
58	O129R	+	+
59	GGAA	-	-
61	IMLTA	-	-
62	ELLM	+	+
64	ILATA	-	-
		Human	Human
	Source/	12 (18)	4 (6.1)
	No. (%)	Camel	Camel
		16 (30.8)	10 (19.2)

strains in 9 profiles. P1 and P2 which contain biochemical reactions of classical *E. coli* isolates, were represented by 24.1% human and 50% camel strains (Table 2). Percentage variation between human and camel strains could be explained by the fact that camel isolates were obtained from apparently healthy animals. However, it has been established that domestic animals are the natural reservoirs of *E. coli* and the uncontrolled release of faeces results in the presence of these bacteria in the environment (Capriole et al., 2005). Pathogenic *E. coli* has been recovered from water, sewage, vegetables and sprout (Fremaux et al., 2008; Miko et al., 2013; Scharlach et al., 2013).

All human and camel strains, in the present study, were urease negative although human isolates were obtained from cases of UTI. It needs further work to detect whether this phenotype offers any pathological advantage to isolates of UTI.

E. coli O157 strains are β -glucuronidase and sorbitol negative. β -Glucuronidase appears to be a confirmed character to differentiate between *E. coli* O157 and non-O157 strains, however, the sorbitol fermentation is more questionable (Leclercq et al., 2001). In the present study, four human isolates (6.1%) were β -glucuronidase negative but sorbitol positive whereas, three camel isolates (11.5%) were sorbitol negative and β -glucuronidase positive. It is worth mentioning that Vitek 2 system gives results of testing for 24 h; some strains of *E. coli* may ferment sorbitol after 48 h of incubation. As for human isolates, being sorbitol positive, throws doubt for confirmation as O157 strains. Furthermore, *E. coli* O157 has not been reported from UTIs in humans. In another study, Leclercq et al. (2001) reported that negativity of β -glucuronidase was fairly frequent (17.9%) among non-O157 serotypes.

In the present study, P4 was positive for malonate and identified from 4 human strains. This is in disagreement with other studies which reported that all *E. coli* tested strains were negative for malonate (Farmer et al., 1985; Ewing, 1986; Leclercq et al., 2001; Koneman et al., 2005), however, Krieg and Holt (1984) reported positive reaction in a range of 0 to 1% in the strains.

The results of the present study suggest that *E. coli* of profile number P12 consisted of four human strains which were negative for ornithine decarboxylase and β -glucuronidase. Other reports indicated that approximately 30% of *E. coli* clinical isolates were ornithine decarboxylase negative (Leclercq et al., 2001; Koneman et al., 2005). P18 and P19 of camel strains did not ferment D-maltose and D-mannose. It was reported that about 2% *E. coli* isolates are D-maltose and D-mannose negative (Koneman et al., 2005).

In the present study, it was demonstrated that 9.6% camel and 21.2% human strains were β -hemolytic. This indicates that some camel isolates could be potential pathogens. Other investigators reported that 10% of *E. coli* isolates from UTI in humans were hemolytic (Bhattacharyya et al., 2015).

Table 3. Contd.

Code	Reagent	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19
56	CMT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57	BGUR	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
58	O129R	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
59	GGAA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	IMLTA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	ELLM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
64	ILATA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Human	H	H	H	H	H	H	H	H	H	H	C	C	C	C	C	C	C
	Camel	H	H	H	H	H	H	H	H	H	H	C	C	C	C	C	C	C
	No. (%)	4(6.1)	4 (6.1)	4 (6.1)	4 (6.1)	4 (6.1)	8(12.1)	4 (6.1)	4 (6.1)	6(9.1)	4 (6.1)	4 (7.7)	2(3.8)	2(3.8)	4 (7.7)	4 (7.7)	4 (7.7)	6(11.5)

Results of the current study show that 50% of camel strains and 25% of human strains displayed biochemical activities of classical *E. coli*. The rest of the strains could not be assigned to a single profile, half of camel isolates were distributed into 7 profiles and three-quarters of human isolates were distributed into 10 profiles. This is interesting as there are no previous studies on biochemical characterization of *E. coli* camel isolates in the study area. All the tested strains should be considered as non-O157 *E. coli*. More work is needed to investigate more *E. coli* strains from sick camels and human UTIs for phenotypic and genotypic characteristics from the study area.

Conclusion

E. coli isolates from community-acquired UTIs in the study area belongs to the group, *non-O157 E. coli* which showed similarity with camel faecal isolates. Biochemical activities indicated that half camel and quarter human strains were classical as non-O157 *E. coli*, the remaining strains displayed deviation in some biochemical

reactions.

The rest of the strains could not be assigned to a single profile, half of camel isolates were distributed into 7 biochemical profiles and three-quarters of human isolates were distributed into 10 profiles.

VITEK 2 identification system for Gram negative bacterial species is helpful in biochemical confirmation of *E. coli* isolates especially for differentiation of *E. coli* O157 and non-O157.

Conflict of Interests

The authors have not declared any conflict of interests.

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