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Isolation and Characterization of Multi Drug Resistant Escherichia Coli from Clinical Sample

Jahanara Utsad[1] Prabhurajeshwar C.[2] Prof. Chandrakanth Revanasiddappa Kelmani[3]*

[1, 2, 3] Medical Biotechnology and Phage Therapy Laboratory, Department of Post Graduate Studies and Research in Biotechnology, Gulbarga University, Gulbarga-585106, Karnataka, India

Abstract – Manhandle and despicable recommending approach of anti-infection agents causes exceptional increment of anti-infection opposition design among the E. coli isolates from clinical sample. This examination was directed to disconnect and recognize multi medicate safe uropathogenic Escherichia coli from urine test of urinary tract contaminated patients by tradition and customary methods. Twenty two (22) UTI urine samples were gathered from patients admitted to close-by healing centers. Portrayal of confined strains were dictated by biochemical tests like Gram recoloring, indole, oxidase, catalase, methyl red, Voges-Proskauer, citrate usage, hemolysis, motility; hydrolysis trial of gelatin and urea; maturation and use trial of glucose, lactose and sucrose. Affectability example of segregates was resolved against some customary and ordinary anti-toxins. From this examination, it was uncovered that 56.75% confines were gram negative. Indole energy was watched for 95.23% of Gram negative disengages. 100% of indole positive isolates were certain for catalase, methyl red, nitrate lessening, motility, and hemolysis, starch maturation on soup medium and starch use on Triple sugar press agar. Cynicism was seen for oxidase, Voges-Proskauer, urease, citrate, and gelatinase test. It was likewise watched that 100% of indole positive disengages gave inspiration on differential media, for example, MacConkey agar and EMB agar. Anti-microbial affectability shows 100% of portrayed strains were multi medicate safe. The discoveries of the investigation proposed that 20 E. coli were distinguished and every one of them were multi medicate safe strains.

Keywords: Escherichia Coli, Antibiotic Susceptibility, Disc Agar Diffusion, Multi Drug Resistant

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INTRODUCTION

E.coli is commensally growing bacteria found in human as well as animals. In humans, they are the major aerobic organism residing in the intestine, typically with around 106 to 109 colony forming units per gram of stool (Akram, et. al., 2007). The organism is also found in soil and water, usually as a result of fecal contamination but the pathogenic variants can cause various types of infection including gastroenteritis, urinary tract infection, meningitis, peritonitis and septicemia (Bannerman, 2003, Barnes, et. al., 2003). Treatment to this infection is complicated due to emergence of multi drug resistance among pathogenic variants. In last 20 years of time span major increases in emergence and spread of multidrug-resistant bacteria and increasing resistance to antibiotic groups, such as fluoroquinolones and certain cephalosporins (Bauer, et. al., 1966).

The β-lactams anti-microbials, in mix with amino glycosides, are among the normally recommended anti-infection agents which are significant piece of experimental treatment. As a result of foolish and pointless use in creating nations, protection from these medications has turned into a noteworthy issue. An element in the rise of multidrug-safe Gram-negative bacilli is the creation of expanded range b-lactamases (ESBLs) and enzymatic alteration of amino glycosides, which are in charge of protection from b-lactams anti-infection agents and amino glycosides, separately (Bentley & Meganathan, 1982). CMY, CTX-M, and NDM sorts of b-lactamase are for the most part in charge of the developing protection from the β-lactams anti-infection agents among *E. coli* (Ewing, 1986). The b-lactam anti-infection agents, particularly the cephalosporin's and b-lactam-b-lactamases inhibitor blends are real medication classes used to treat contaminations caused by *E. Coli*. (Akram, et. al., 2007). Among *E. coli*, b-lactamase generation remains the major contributing variable to b-lactam obstruction. Expanded range b-lactamases are one of the real wellsprings of protection from oxyimino-cephalosporins in Enterobacteriaceae (Bannerman, 2003). The majority of ESBLs are mutants of TEM and SHV catalysts, yet CTX-M chemicals are the recently developing ESBLs (Barnes, et. al., 2003) and are progressively common worldwide among E.coli microscopic organisms. The CTX-M proteins

are wide gathering with in excess of 30 alleles classified into five particular phylogenetic gatherings, advanced in view of hereditary escape and transformation of the chromosomal b- lactamase qualities of *Kluyvera spp* (Bannerman, 2003, Barnes, et. al., 2003). The CTX-M family, first portrayed in 1992 (Bauer, et. al., 1966), is known as most predominant non-TEM, non-SHV ESBL among Enterobacteriaceae.

It is recognized as a quickly developing group of ESBLs that specifically want to hydrolyze cephotaxime and a large portion of them are dynamic against ceftazidime (Bentley & Meganathan, 1982). Assist the frequency of Urinary tract contaminations (UTI) by ESBL delivering *E. coli* was observed to be the most elevated in India (60%) trailed by Hongkong (48%) and Singapore (33%) (Chakraborty, et. a., 2011b). Past investigations from India have announced ESBL creation shifting from 28% to 84% (Chakraborty, et. al., 2011).

In general commonness of ESBL makers was found to fluctuate in various topographical areas and in various organizations. CTX-M compounds have been the dominating ESBLs in Argentina for >10 years (Cheesbrough, 1985) and have pervasiveness in numerous parts of the world (Barnes, et. al., 2003, Duguid, 1996)

Including Europe (Eaton, et. al., 1995, Ewing, 1986) Consequently in the present investigation we are transcendently focusing on commonness of CTX-M ESBL delivering E.coli in our area.

By and large *E. coli* strains don't cause disease (Reid, et. al., 2001), anyway hurtful strains can cause gastroenteritis, urinary tract pollutions, neonatal meningitis, hemorrhagic colitis, and Crohn's illness. Typical signs and signs consolidate outrageous stomach fits, detachment of the insides, hemorrhagic colitis, regurgitating, and a portion of the time fever. In rare cases, dangerous strains are in like manner accountable for entrail decay (tissue destruction) and puncturing without progressing to hemolytic-uremic confusion, peritonitis, mastitis, septicemia, and Gram-negative pneumonia. To a great degree young children are more helpless against make genuine illness, for instance, hemolytic uremic issue, regardless, solid individuals of all ages are in threat to the genuine results that may rise due to being polluted with *E. coli.*

A couple of strains of E. coli for example 0157:H7 can convey Shiga poison (named a bioterrorism master). This toxic substance causes less than ideal destruction of the red platelets, which by then stop up the body's isolating structure, the kidneys, causing hemolytic-uremic turmoil (HUS).Unlike for the most part *E. coli* that regularly live in the gut, the Shiga toxin that causes ignitable responses in target cells of the gut (the wounds the toxic substance deserts are the inspiration driving why shocking free guts is a reaction of a Shiga toxin making *E. Coli* infection).In some exceptional cases (normally in kids and the elderly) Shiga toxin making *E. Coli* malady may provoke hemolytic uremic issue (HUS), which can cause kidney dissatisfaction and even passing. Indications of hemolytic uremic issue consolidate lessened repeat of pee, apathy, and whiteness of cheeks and inside the lower eyelids. In 25% of HUS patients, snares of tangible framework happen, which in this way causes strokes on account of little bunches of blood which stop in vessels in the psyche. This causes the body parts controlled by this zone of the psyche not to work suitably. Moreover, this strain causes the improvement of fluid (since the kidneys don't work), inciting edema around the lungs and legs and arms. This extension in fluid advancement especially around the lungs hinders the working of the heart, causing a development in circulatory strain.

Uropathogenic *E. coli* (UPEC) is one of the essential drivers of urinary tract diseases. It is a bit of the customary vegetation in the gut and can be exhibited from various perspectives. Particularly for females, the heading of wiping after crap (wiping back to front) can provoke fecal debasement of the urogenital gaps. Butt-driven intercourse can similarly carry this bacterium into the male urethra, and in changing from butt-driven to vaginal intercourse, the male can in like manner familiarize UPEC with the female urogenital framework. For more information, see the databases toward the complete of the article or UPEC pathogenicity.

MATERIALS AND METHODS

1. Isolation and identification of *E.coli*

In this study 150 strains of E.coli from 170 different clinical samples like urine, stool, blood, pus etc were isolated. The clinical samples were collected from hospitals and diagnostic centres in Kalaburagi region over a period of three months in 2013. Identification was done by culture on EMB agar. Isolation of strains was done by conventional morphological, cultural and biochemical characterization. Standard strain of *E.coli* MTCC 443 was obtained from Medical and Phage Therapy Laboratory, department of Biotechnology, Gulbarga University, Kalaburagi.

2. Biochemical tests

Catalase test

Both on tube and slide catalase trial of isolates was performed by (MacFaddin JF, 2000) utilizing 3-6% hydrogen peroxide (H2O2). *E. faecalis* ATCC 29212 and *E. coli* ATCC 25922 were taken as negative and positive control, individually.

Oxidase test

Oxidase trial of isolates was performed by channel paper technique agreeing Snell JJS et al. 1999 utilizing 1% N, N, N', N'- tetramethyl-pphenylenediamine dihydrochloride arrangement. E. coli ATCC 25922 were taken as negative and positive control, individually.

Indole test

Indole test was finished by adding Kovac's reagent to culture media as indicated by Cheesbrough, 1985. *E. coli* ATCC 25922 and Pseudomonas aeruginosa 27853 were taken as positive and negative control strains respectievely.

MR-VP test

Both MR and VP tests were finished by Cheesbrough, 1985 utilizing 0.7 gm% Peptone blend, 0.5 gm% Potassium Phosphate and 0.5 gm% Dextrose for culture media supplements. 0.4% Methyl red arrangement, 5% alpha napthol in supreme liquor and 40% sodium hydroxide arrangement were utilized as marker for the said tests. *E. coli* ATCC 25922 and Enterobactor aerogenes ATCC 1304 were taken as positive and negative control strains for this test individually.

Citrate Utilization

Citrate use test was performed by the standard technique for Simmons J, 1960 utilizing, 0.1 gm% ammonium di-hydrogen phosphate, 0.5 gm% NaCl, 0.02 gm% MgSO4, 0.008 gm% Bromothymol blue, 0.1 gm% di-potassium phosphate, 0.2 gm% Sodium citrate and 1.5 gm% Bacteriological agar. *E. coli* ATCC 25922 and Enterobactor aerogenes ATCC 1304 strains were taken as positive and negative control separately for these tests.

Gelatinase test

Gelatin hydrolysis test was finished concurring the strategy for Chakraborty SP et al. 2011b. *E. coli* ATCC 25922 strains were taken as positive control and negative control separately.

Urease test

Urease test for bacterial disconnects was finished by the technique Chakraborty SP *et al.* 2011b. Media were set up with urea 2 gm %, Agar 1.5 gm %, Nacl 0.5gm%, KH2PO4 0.2 gm% and phenol red 0.0012 gm% in incline position. *E. coli* ATCC 25922 and Proteus vulgaris ATCC 13315 were taken as positive and negative control strains for the test.

Triple sugar Iron (TSI) tests

Triple sugar Iron test was performed by Vanderzant C and Splitt stresser DF, 1992. All the bacterial confines were immunized on disinfected Triple Sugar Iron Agar tubes containing Peptone blend 2 gm%, Sucrose 1 gm%, Beef extricate 0.3 gm%, Dextrose 0.1 gm%, Sodium thiosulphate 0.03 gm%, Lactose 1.0 gm%, Sodium Chloride 0.5 gm%, Yeast Extract 0.3 gm%, Ferrous Ammonium Citrate 0.025 gm% and Bacteriological Agar 1.2 gm%. *E. coli* ATCC 25922 strains was considered as positive control.

Carbohydrate aging tests

Starch maturation trial of clinical disconnects were performed by the technique for Cheesbrough, 1985. In a nutshell, Isolates were vaccinated on Phenol red Dextrose stock (Casein peptone 1.0 gm%, NaCl 0.5 gm%, Dextrose 0.5 gm%, Phenol red 0.0018 gm %) with Durham tubes for glucose maturation think about. For Sucrose and Lactose aging confines were immunized on Phenol red Sucrose broth(Casein peptone 1.0 gm%, NaCl 0.5 gm%, Sucrose 0.5 gm%, and Phenol red 0. 0018 gm%) and Phenol red Lactose stock (Casein peptone 1.0 gm%, NaCl 0.5 gm%, Lactose 0.5 gm%, and Phenol red 0.0018 gm%). For every one of the three aging tests stock medium were brooded for 48 hours in an oxygen consuming hatchery. Positive outcome was noted by change of media shading from red to yellow and creation of gas on Durham's tube. *E. coli* ATCC 25922 and *Shigella flexneri* ATCC 12022 were taken as positive and negative control strains individually.

Motility test

Motility trial of confines was performed by Cheesbrough, 1985. Quickly, semi strong agar was readied utilizing hamburger separate 0.3%, pancreatic process of casein-1.0%, Nacl 0.5% and Agar-0.4%. *E. coli* ATCC 25922 S. aureus ATCC 25923 and were taken as positive and negative control, individually.

Nitrate Reduction test

Nitrate reduction tests were finished by "Snell EE and Wright LD, 1941". To sum things up, Nitrate soup was readied (casein Peptone 0.5 gm%, Beef Extract 0.3 gm%, Potassium Nitrate 0.1 gm%, Galactose 0.5 gm% and Disodium Phosphate 0.25 gm %) with Durham tubes (gas gatherer) in it. 0.8 gm% sulphanilic corrosive (broke up in 5M acidic corrosive) and 0.6 gm% alpha naphthole (disintegrated in 5M acidic corrosive) and Zinc clean were all the while added to those societies. Acinetobacter calcoaceticus ATCC 19606 and E. coli

ATCC 25922 were taken as negative and positive control strains separately.

Hemolysis on blood agar

Hemolysis trial of confined microscopic organisms was performed by immunizing them into blood agar plates (Nutrient agar 2.4 gm% and 5 % Sheep blood). Plates were brooded at 37°C for 24 hr. A clearing zone encompass the bacterial settlement was watched and recorded (Bannerman TL, 2003). *E. coli* ATCC 23509 and *E. coli* ATCC 25922 were taken as negative and positive control, separately.

RESULT AND DISCUSSION

1. Isolation

150 strains of *E.coli* were isolated from 170 different clinical samples and its identified by conventional methods. Highest number of strains was isolated from urine and least from blood sample as shown in table No 1.

Sl. No	Clinical samples	No. of strains isolated	% of resistance
1	Urine	70	46.6%
2	Stool	59	39.35%
3	Blood	5	4%
4	Pus	6	3.25%
5	Others	10	6.6%

Table 1: Distribution of E.coli isolates in clinical samples and percentage of resistance.

2. Identification and screening of *E. coli*

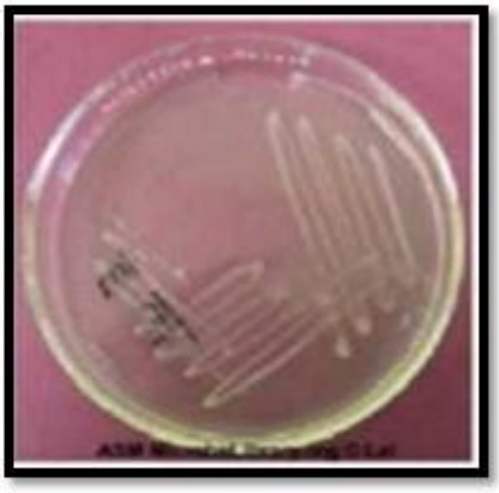
The greater part of the clinical isolates was observed to be *E. coli.* The source of all clinical separates is appeared in figure 1. Out of the 150 clinical isolates of *E. coli*, 40.08% were seen to be ESBL positive, 16.16% disengages were MβLs positive, and 17.24% were both ESBL and MβLs positive. The staying 26.50% were viewed as non ESBLs and MβLs.

Diversity of ESBLs and MβLs

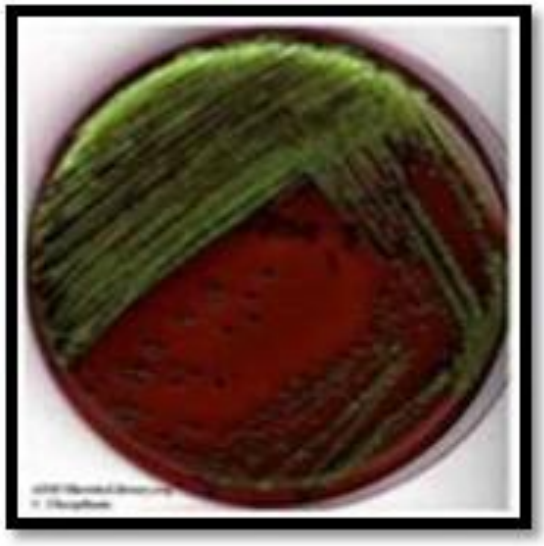
Results acquired in the present examination demonstrated that TEM-type ESBLs (bla TEM-I, bla TEM- 2, bla TEM-50) were found in around 57% of the isolates. The commonness of SHV-type, CTX-M- sort and OXA write ESBLs gave off an impression of being 29.03, 11.82 and 2.15%, separately. Among the MβLs, the recurrence of dissemination of NDM-1, IMP-1, VIM-1 and KPC-type was 37.39, 21.33, 18.66 and 22.66%, separately. The point by point dispersion of ESBLs+MβLs is outlined.

3. Biochemical test of isolates

The clinical isolates were distinguished utilizing standard biochemical tests. Purification of bacterial culture were finished utilizing single colony isolation method on Nutrient agar containing 10% sodium chloride, showed a few kinds of provinces. From the examination it was observed that, 56.75% separates were Gram negative and 43.25% isolates were Gram positive; 95.23% of gram negative isolates are indole positive. 100% of indole positive segregates were Catalase positive, MR positive, Nitrate diminishment test positive, Carbohydrate maturation (Glucose, Lactose, Sucrose) test positive and furthermore positive for starch use with gas creation on TSI. All Indole positive isolates were negative for Oxidase test, VP test, Urease test, Citrate test and Gelatinase test. It was additionally uncovered that 100% of Indole positive segregates were uropathogenic for giving hemolytic movement (60 % beta hemolytic and 40 % gamma hemolytic) on blood agar and are on the whole motile (Table 1a and Table 1b)(this is mentioned below). On MacConkey agar w/o Crystal violet and EMB agar 100% of Indole positive isolates gave red-pink shading colony and green with metallic sparkle settlement respectively. Subsequently among 37 clinical segregates 20 (54.05%) isolates were affirmed to be uropathogenic *Escherichia coli* (UPEC).



Nutrient agar inoculated with *Escherichia coli* demonstrating growth with whitish colonies.



EMB agar inoculated with *Escherichia coli* demonstrating growth with green-metallic sheen colonies

Table 1a: Standard biochemical tests of clinical isolates, collected from urine sample of UTI patient. ND =

Tests are not done, + ve = tests are positive, - ve = tests are negative.

Sample	Isolates	Gram Staining	Indole	Oxidase	Catalase	MR	VP	Urease	Citrate utilization
S1	S1a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S1b	+ ve	ND	ND	ND	ND	ND	ND	ND
S2	S2a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S3	S3a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S3b	+ ve	ND	ND	ND	ND	ND	ND	ND
S4	S4a	+ ve	ND	ND	ND	ND	ND	ND	ND
	S4b	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S5	S5a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S5b	+ ve	ND	ND	ND	ND	ND	ND	ND
S6	S6a	- ve	- ve	ND	ND	ND	ND	ND	ND
	S6b	+ ve	ND	ND	ND	ND	ND	ND	ND
S7	S7a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S8	S8a	+ ve	ND	ND	ND	ND	ND	ND	ND
	S8b	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S9	S9a	+ ve	ND	ND	ND	ND	ND	ND	ND
	S9b	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S10	S10a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S11	S11a	+ ve	ND	ND	ND	ND	ND	ND	ND
	S11b	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S12	S12a	+ ve	ND	ND	ND	ND	ND	ND	ND
	S12b	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S13	S13a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S13b	+ ve	ND	ND	ND	ND	ND	ND	ND
S14	S14a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S14b	+ ve	ND	ND	ND	ND	ND	ND	ND
S15	S15a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S16	S16a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S16b	+ ve	ND	ND	ND	ND	ND	ND	ND
S17	S17a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S18	S18a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S18b	+ ve	ND	ND	ND	ND	ND	ND	ND
S19	S19a	+ ve	ND	ND	ND	ND	ND	ND	ND
	S19b	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
S20	S20a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve
	S20b	+ ve	ND	ND	ND	ND	ND	ND	ND
S21	S21a	+ ve	ND	ND	ND	ND	ND	ND	ND
S22	S22a	- ve	+ ve	- ve	+ ve	+ ve	- ve	- ve	- ve

Table 1b: Standard biochemical tests of clinical isolates, collectedfrom urine sample of UTI patient. ND = Tests are not done, + ve = tests are positive, - ve = tests are negative. A/AG = Acid slain and acid butt with gas production.

Sample	Isolates	Nitrate reduction	Gelatinase	TSI	Carbohydrate fermentation		Hemolysis		Motility
S1	S1a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S1b	ND	ND	ND	ND	ND	ND	ND	ND
S2	S2a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S3	S3a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S3b	ND	ND	ND	ND	ND	ND	ND	ND
S4	S4a	ND	ND	ND	ND	ND	ND	ND	ND
	S4b	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S5	S5a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S5b	ND	ND	ND	ND	ND	ND	ND	ND
S6	S6a	ND	ND	ND	ND	ND	ND	ND	ND
	S6b	ND	ND	ND	ND	ND	ND	ND	ND
S7	S7a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S8	S8a	ND	ND	ND	ND	ND	ND	ND	ND
	S8b	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S9	S9a	ND	ND	ND	ND	ND	ND	ND	ND
	S9b	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S10	S10a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S11	S11a	ND	ND	ND	ND	ND	ND	ND	ND
	S11b	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S12	S12a	ND	ND	ND	ND	ND	ND	ND	ND
	S12b	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S13	S13a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S13b	ND	ND	ND	ND	ND	ND	ND	ND
S14	S14a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S14b	ND	ND	ND	ND	ND	ND	ND	ND
S15	S15a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S16	S16a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S16b	ND	ND	ND	ND	ND	ND	ND	ND
S17	S17a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S18	S18a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S18b	ND	ND	ND	ND	ND	ND	ND	ND
S19	S19a	ND	ND	ND	ND	ND	ND	ND	ND
	S19b	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
S20	S20a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile
	S20b	ND	ND	ND	ND	ND	ND	ND	ND
S21	S21a	ND	ND	ND	ND	ND	ND	ND	ND
S22	S22a	+ ve	- ve	A/AG	+ ve	+ ve	+ ve	+ ve	Motile

CONCLUSION

From our study it can be concluded that twenty E. coli strains was successfully characterized by biochemical methods.

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Corresponding Author

Prof. Chandrakanth Revanasiddappa Kelmani*

Medical Biotechnology and Phage Therapy Laboratory, Department of Post Graduate Studies and Research in Biotechnology, Gulbarga University, Gulbarga-585 106, Karnataka, India

E-Mail – ckelmani@gmail.com