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Characterization of Haemolytic Escherichia coli

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Abstract- A total of 23,007 clinical samples, 2,001 bacterial isolate were identified as *Escherichia coli*, of which 205 strains were haemolytic. On biotyping, indole test was found positive in 99.52% strains. Glucose, maltose, trehalose were fermented by all strains. Dulcitol was fermented by 80.98% strains. Raffinose, sucrose, adonitol and inositol were fermented by 61.96%, 49.76%, 35.13% and 34.15% strains respectively. Lysine, ornithine and arginine were decarboxylated / hydrolyzed by 90.74%, 82.44% and 22.44% strains respectively. Serotypability was 82.9% and 51 serogroups were detected. 35.14% belonged to 3 serotypes (O6, O2 & O4). Resistogram typing showed that most of the strains were resistant to Boric acid, Malachite green and Cetrimide. These strains fell into 57 different resistotypes. Antibiogram revealed a higher degree of multiple drug resistance (53.75%).

Index Terms- Antibiogram, Biotyping, Haemolytic *Escherichia coli*, Resistotyping, Serotyping

I. INTRODUCTION

Escherichia coli (E.coli) the most common enteric organism causing extra-intestinal infections in man and tops the list, particularly of urinary tract pathogens. E.coli strains isolated from the case of intestinal and extra-intestinal diseases are endowed with certain traits considered of importance of virulence. Virulence of E.coli is multifactorial. One of these is the ability to produce haemolysin. The significantly higher association of haemolytic E.coli with extra intestinal infection than with normal flora has led to the consensus that haemolytic E.coli strains are more likely to cause disease than non-haemolytic E.coli [1, 2]. Haemolysin contributes not only to resistance of E.coli to intracellular killing, but also to resistance to the bactericidal activity of serum [3, 4]. Various reports not only confirm the role of haemolytic E.coli in the etiopathogenesis of pyelonephritis but also substantiate the role of haemolysin as a potential virulence factor in urinary tract infection [5]. Therefore, keeping in mind the reported incidence of haemolytic E.coli in clinical samples and their association with other virulence factors in causing various infections, the present study has been undertaken. The study was aimed to find out the incidence of haemolytic E.coli in different clinical samples and subjected them to Biotyping, Serotyping, Resistotyping and Antibiotic Sensitivity pattern.

II. MATERIAL AND METHODS

The study was conducted over a period of fourteen months. During the study, 23,007 samples were submitted in the Microbiology department for culture sensitivity and were processed on MacConkey agar and Blood agar by the conventional methods [6]. All the 205 strains of haemolytic E.coli thus obtained were stock cultured till studied. These strains were subjected to Biotyping, Serotyping, Resistotyping and Antibiotic Sensitivity.

A. Biotyping

For biotyping the technique of Edward and Ewing (1972) was used. Various biochemical tests performed were fermentation tests, decarboxylase / dehydrolase activity tests and the tests for enzymes.

B. Serotyping

All the strains were sent to National Salmonella and Escherichia Serotyping Centre, Central Research Institute (CRI) Kasauli (Himachal Pradesh) for serotyping against somatic O antigen.

C. Resistotyping

Resistotyping was done by the method of Elek and Higney (1970). Various chemicals used were Acriflavin (A), Boric acid (B), Copper sulphate (C), Malachite green (D) and Cetrimide (E) in the different concentration of 0.090%, 3.0%, 0.7%, 0.003% and 0.05% respectively in water (W/V). The stock solutions were kept in refrigerator and were used within a week. The inoculum size was one loop full containing approximately 1000 organisms.

D. Antibiotic Sensitivity

It was done by Kirby-Bauer disk diffusion technique [9] against ampicillin (25ug), cefaparazone (30ug), ciprofloxacin (5ug), co-trimoxazole (25ug), gentamycin (10ug), nalidixic acid (30ug), netilmicin (10ug) and norfloxacin (10ug).

III. RESULTS

Out of 205 haemolytic strains of *E.coli*, 181 were from urine, 10 from vaginal /cervical swabs, 7 from stool and 7 from pus and other samples. The biochemical reactions of all strains were as in Figure1 and Table 1. On serotyping, 51 different serogroups were found prevalent on the basis of somatic O antigen. The incidence of different serotypes and common serogroups in different samples

is shown in Table 2 and 3. All the strains were resistotyped with five chemicals coded as A, B, C, D and E [Fig. 2]. These strains fell into 57 different resistotypes. The frequency and distribution of these strains with respect to their resistotypes is shown in Table 4. Antibiotic sensitivity testing revealed 53.75% strains to be multidrug resistant, 6.83% strains were sensitive to all the eight agents [Fig. 3].

IV. DISCUSSION

The present study was aimed at finding out the incidence of haemolytic *E.coli* from different clinical specimens and their further characterization by biotyping, serotyping, resistotyping and antibiotic sensitivity. There is no study carried out on these lines exclusively for haemolytic *E.coli*, though the studies on *E.coli* in general are available in plenty. Therefore, the results in the present study are being compared with these of *E.coli* reports in general only.

A total of 205 strains were identified as haemolytic *E.coli*. As far as the biotyping is concerned, the indole test and methyl red test were found to be positive in 99.52% and 100% strains respectively. Nitrate reduction test was positive in 100% strains. Whereas, Tewari and Aggarwal (1982) reported in their study, the positivity rate of these three reactions as 100%. Voges Proskauer test and urease test were negative in all strains. Like Tewari and Aggarwal (1982), in our study also, all the strains have been found to ferment glucose, maltose, trehalose and mannitol. Regarding the fermentation of arabinose, adonitole, inositol, raffinose and sucrose, 85.37%, 35%, 34%, 61.96% and 49.76% strains have been found positive in the present study whereas others reported a positive rate of 99%, 5%, 1%, 64.9%, 91.4% respectively [10-12]. As far as the fermentation of xylose and lactose is concerned, in our study, 98.54% strains were found to ferment these two sugars whereas Forbes *et al.,* (2002) and Tewari and Aggarwal (1982) observed that 95%, 95%, 70.1% and 94.6% of *E.coli* strains to ferment xylose and lactose respectively. Kalmansen *et al.,* (1975) reported that dulcitol fermenting *E.coli* strains are significantly more virulent than the others and have shown the correlation between the urovirulence of *E.coli* and dulcitol fermentation. In the present study, 80.98% (166) strains have been found to ferment dulcitol as compared to Kaur *et al.,* (1991) and Forbes *et al.,* (2002) who reported 71.1% and 60% of their strains to ferment dulcitol. Only 3.9% strains were anaerogenic in our study, as compared to others who reported 7.7% strains to be anaerogenic [10]. In the present study, 90.74%, 82.44% and 22.44% strains were found to decarboxylate / hydrolyse lysine, ornithine and arginine respectively. The studies conducted by other workers reported the positivity rate ranged from 90% - 100%, 65% - 67% and 17% - zero respectively [10-12].

In *E.coli*, so far more than 173 serotypes of O antigens, 103 serotypes of K antigens and 75 serotypes of H antigens have been recognized [15]. In the present study, only 170 (82.9%) haemolytic *E.coli* strains are serotypable, 4.39% and 12.69% strains were untypable and rough respectively. The higher percentage of these rough strains could be either because of the present study involved only one morphological type of strains (haemolytic *E.coli*) or it may be due to different geographical distribution or due to untreated cases of *E.coli* infection [16] though in present study, history of treatment was not considered. Husain *et al.,* (1995) reported only 6.1% strains as rough strains in their study. Hughes *et al.,* (1982) and Goldwater and Bettelheim (2000) reported 50% *E.coli* strains of O18 and O75 and O111 serogroups to be haemolytic in UTI and Haemolytic Uremic Syndrome cases respectively whereas in present study, only 2.35% strains belonged to serotype O18 and no strain fell in serogroup O75 and O111 respectively. Out of 181 strains from urine, only 40% strains belonged to classical serogroups as compared to Siegfried *et al.,* (1994) who observed 35.4% and 25% strains belonged to classical serogroups from lower and upper urinary tract infections respectively. Resistogram is considered better method than antibiogram for typing an organism. This may also give epidemiologically valuable information. Elek and Higney (1970) identified 65 serotypes for 200 strains of *E.coli* isolated from UTI. In the present study, some strains were serologically untypable (4.39%) but these could be typed by resistotyping. Similar correlation between resistotyping and serotyping has also been shown by Elek and Higney (1970). The antibiogram pattern of haemolytic *E.coli* isolated in the present study showed multidrug resistance (53.75%). Only 6.83% strains were sensitive to all the 8 agents tested. About 13% strains were resistant to single drug and 23.43% strains were resistant to two drugs. On the whole, cefaperazone (90.25%), netilmycin (83.9%), ciprofloxacin (74.15%), gentamycin (72.20%) and norfloxacin (71.71%) have shown good in-vitro efficacy. More than 80% of isolates of *E.coli* were reported to be resistant to ampicillin [10,19] which is similar to that found in the present study (84.39%). The percentage of cotrimoxazole, nalidixic acid and gentamycin resistant strains in our study is 76.10%, 53.66% and 27.80% respectively, meaning thereby that haemolytic *E.coli* strains showed greater degree of drug resistance than non-haemolytic *E.coli* isolates [10,14,19,20].

V. CONCLUSIONS

The incidence of haemolytic *E.coli* is 10.24% among *E.coli* isolates. Biochemically, these strains show all most similar reactions as non-haemolytic *E.coli*. Only 82.9% of the strains were typable serologically with incidence of rough strains being on the higher side. In the present study, 40% strains from urine belonged to classical groups. Another contrast is that only 2.35% strains belonged to serotype O18 and none to O75 and O111 serogroups as compared to other studies who reported 50% *E.coli* strains of O18, O75 and O111 to be haemolytic. The resistotyping is a useful epidemiological marker as 57 resistotypes were encountered in a study of 205 strains. Another feature revealed from the study is that the antibiogram of these strains showed greater degree of resistance than non-

haemolytic *E.coli*. So haemolytic *E.coli* characters are similar in some aspects with the non-haemolytic *E.coli* and differ widely in others.

Table 1: Carbohydrate fermentation and decarboxylase / dehydrolase activity of hemolytic *Escherichia coli* (n=205)

S. No.	Carbohydrates / Amino acid	Positive Number	Percentage
1.	Adonitol	72	35.13 %
2.	Arabinose	175	85.37 %
3.	Dulcitol	166	80.98 %
4.	Glucose	205	100 %
5.	Inositol	70	34.15 %
6.	Lactose	202	98.54 %
7.	Maltose	205	100 %
8.	Mannitol	205	100 %
9.	Raffinose	127	61.96 %
10.	Sucrose	102	49.96 %
11.	Trehalose	205	100 %
12.	Xylose	202	98.54 %
13.	Arginine	46	22.44 %
14.	Lysine	186	90.74 %
15.	Ornithine	169	82.44 %

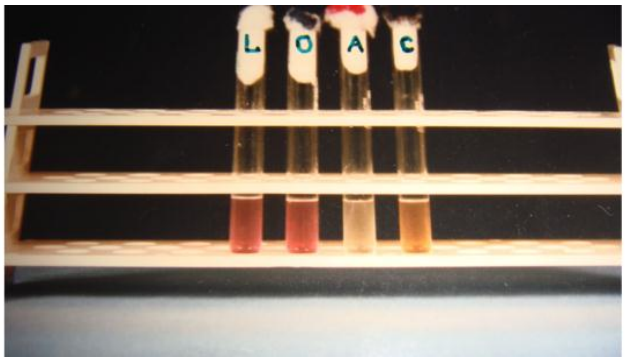

Figure 1: Decarboxylation of amino acids. L – Lysine, O – Ornithine, A – Arginine, C – Control

Table 2: Incidence of serotypes in haemolytic *Escherichia coli* strains (n=205)

Serotypes	Positive strains	Percentage
O6	38	18.54%
O2	17	8.30%
O4	17	8.30%
O138	7	3.42%
O106	6	2.93%
O35, O56, O130	5 each	2.44%
O18, O154	4 each	1.96%
O1, O29, O36, O48, O99, O102	3 each	1.47%
O24, O32, O69, O71, O100, O107, O141, O155, O161	2 each	0.98%
O8, O10, O13, O17, O20, O21, O40, O41, O51, O58, O59, O64, O77, O92, O101, O105, O108, O112, O116, O117, O124, O132, O142, O152, O153, O160	1 each	0.49%
Rough	26	12.69%
Untypable	9	4.39%

Table 3: Sample wise serotypes of haemolytic *Escherichia coli* (n=205)

S. No.	Source	No. of strains (%)	No. of different serogroups	Commonest serogroups (No. of strains of each)
1.	Urine	181 (88.3%)	46	O6 (34), O4 (16), O2 (15), O138 (6), O56 (5), O18, O35, O106 (4 each), O29, O48, O99, O102, O130, O154 (3 each), O1, O36, O69, O71, O100, O141, O155, O161 (2 each) & Others (1 each)
2.	Vaginal / Cervical swab	10 (4.9%)	6	O6 (3), O130 (2), O102, O35 and O59 (1 each)
3.	Stool	7 (3.4%)	6	O107 (2), O2, O6, O105, O142 and O154 (1 each)
4.	Pus & Others	7 (3.4%)	7	O4, O24, O32, O36, O106, O116 and O138 (1each)

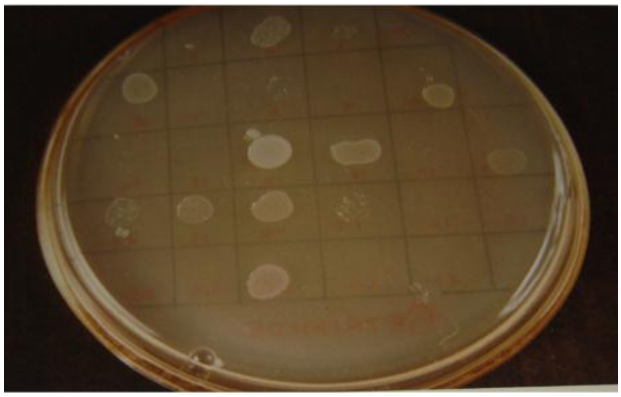

Figure 2: Resistogram pattern to cetrimide. Confluent growth – Resistant, No growth – Sensitive, Discrete colonies or more than ten colonies – Intermediate

Table 4: Resistogram typing of 205 strains of haemolytic *Escherichia coli*

Resistogram Types[1]	No. of Strains
B D E	26
B D	22
D	21
(B) D (E)	13
A B D	12
(B) (D) E	9
B C E	6
D E, B E	5 each
(A) (B) D, (B) (D) (E), C, B, (C) D E	4 each
(A) (B) (C) (D), B (C) D (E), (A) B D E, A C E, B C D	3 each
B (D) (E), A B C D E, A B (C) (D) E, (A) (B) C D E, E, (A) (B) (C) D, (A) B (C) D, (A) (B) (D) E, C D, (A) C D, A E, B C, (A) C D (E) (B) (D), (A) (D) E, (A) (B) (D)	2 each
(A) B (C) (D) (E), (A) (B) (C) (D) (E), (A) (B) (C) D E, (C) (D), C E, (A) C, (A) (E), A (B), (C) D, B C D (E), (A) (B) D (E), B C (D) (E), (B) C D (E), (A) (C) D E, B (C) (D) (E), (A) C (D) (E), (A) (B) D E, (A) B (D) E, (B) (C) D (E), (A) D (E), (A) (B) C, (B) (C) D, C (D) E, C D (E)	1 each

* Resistant to chemical, ( ) - Intermediate resistant to chemical, A - Acriflavin, B - Boric acid, C - Copper sulphate, D - Malachite green, E - Cetrimide

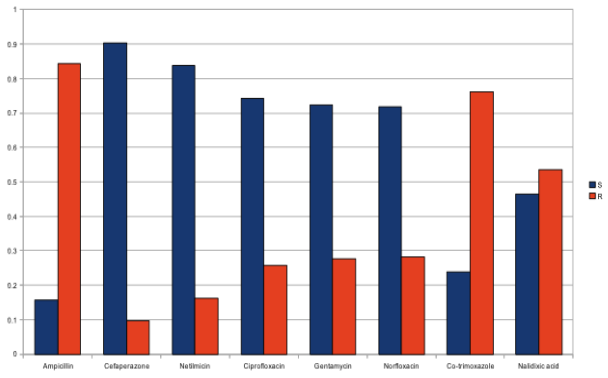

Figure 3: Susceptibility pattern of 205 haemolytic *Escherichia coli* strains

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