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RESEARCH ARTICLE

SIGNIFICANCE OF HAEMOLYTIC *ESCHERICHIA COLI* AMONG CLINICAL ISOLATES

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ABSTRACT

Background: *Escherichia coli* (*E. coli*) is the most common enteric organism causing extra-intestinal infections in man, particularly of urinary tract infection.
Objective: To find out the incidence of haemolytic *E. coli* among different clinical samples and to study their antibiogram.
Methods: All the 23,007 specimens submitted in the department of Microbiology for culture and sensitivity testing were subjected to study. The samples were processed on MacConkey agar and blood agar by conventional methods. Haemolytic *E. coli* were identified on the basis of colony morphology and confirmed by their biochemical characters. These haemolytic strains were further subjected to antibiotic sensitivity by Kirby-Bauer disc diffusion technique.
Results: Out of 23,007 samples, 6,209 bacterial isolates were obtained. Of these, 2,001 were identified as *E. coli.* Amongst these, 205 strains were haemolytic. In urine, the most frequently processed sample, 46.85% of the isolates were *E. coli*. Out of these, 13.97% were haemolytic *E. coli*. The incidence of these strains were found to be 10.10%, 4.29%, 1.68%, and 1.36% from other samples viz vaginal/cervical discharge, faeces, pus and others respectively. A total of 85 (80.18%) haemolytic *E.coli* strains were isolated from females of age group 21 to 40 years. Marked resistance to ampicillin (84.39%), co-trimoxazole (76.09%) and nalidixic acid (53.65%) was observed among these strains.
Conclusion: Haemolytic *E. coli* showed a considerable degree of multiple drug resistance. These organisms are endowed with extra virulence factor, so there is need to have greater awareness to this effect and such cases should be treated more energetically.

INTRODUCTION

Since the discovery of *Escherichia coli* (*E. coli*), many workers have studied haemolytic activity of this organism and have reported that haemolytic strains are more virulent than non-haemolytic strain (Lovell and Rees, 1960; Grover *et al*, 2013). The haemolysin production is considered as one of the virulence factors of *E. coli* and it has been reported that presence of haemolysin is more common in *E. coli* isolated from extra intestinal infections than in those isolated from faeces (Cavalieri *et al*, 1948). Studies have shown an association between haemolytic *E. coli* and fulminant infections in man (Vanden *et al*, 1982; Akulwar *et al*, 1997; Goldwater and Bettelheim, 2000). Therefore, it was decided to find out the incidence of haemolytic *E. coli* amongst *E. coli* isolated from various clinical samples and its antibiotic sensitivity pattern.

MATERIALS AND METHODS

The present study was conducted in the department of Microbiology in tertiary care hospital over a period of 14 months. During the study, a total of 23,007 samples were submitted in the department for culture and sensitivity. These comprised of urine (12,941), blood (2,968), pus (2,679), throat swabs (826), stool (469), vaginal / cervical swabs (411), sputum (294), CSF (188), aural swabs (56), nasal swabs (54), followed by others (2,121) that included various body fluids (Table 1). All the samples were processed on MacConkey agar

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and blood agar by conventional method (Collee *et al,* 1996). Haemolytic *E. coli* were identified on the basis of colony morphology and confirmed by their biochemical characters (Collee *et al,* 1996). Confirmed *E. coli* strains showing haemolysis on blood agar plates were also subjected to antibiotic sensitivity by Kirby-Bauer disk diffusion technique. Clinical and Laboratory Standard Institute (CLSI) reference strain, *Escherichia coli* ATCC (American Type Culture Collection) 25922, was also included as control strain (NCCLS, 2003).

RESULTS

Out of 23007 samples, 6209 bacterial isolates were obtained. Among these isolates, 2001 were identified as *E. coli.* From amongst these 2,001 *E. coli* strains, 205 (10.24%) were identified as haemolytic strains. The *E. coli* isolates from urine, vaginal / cervical swabs, stool, pus and other specimens were 1295, 99, 163, 297, and 147 respectively. The incidence of haemolytic *E. coli* among these samples was found to be 13.97%, 10.10%, 4.29%, 1.68%, and 1.36% respectively (Table 1).

Antibiotic sensitivity: Most of the bacteria were sensitive to cefoperazone (90.24%), netilmicin (83.91%), ciprofloxacin (74.15%), norfloxacin (71.71%) and gentamicin (72.20%). Five strains (2.44%) were resistant to all these drugs tested while 14 strains (6.83%) were found to be sensitive to all the drugs tested (Table 3).

Table 3 Susceptibility pattern of haemolytic *Escherichia coli* strains (n=205)

S. No.	Antibiotics	Resistant strains (%)	Sensitive strains (%)
1.	Ampicillin	173 (84.39)	32 (15.60)
2.	Cefoperazone	20 (9.75)	185 (90.24)
3.	Ciprofloxacin	53 (25.85)	152 (74.14)
4.	Co-trimoxazole	156 (76.09)	49 (23.90)
5.	Gentamicin	57 (27.80)	148 (72.19)
6.	Nalidixic acid	110 (53.65)	95 (46.34)
7.	Netilmicin	33 (16.09)	172 (83.90)
8.	Norfloxacin	58 (28.29)	147 (71.70)

DISCUSSION

The present study was aimed at finding out the incidence of haemolytic *E. coli* from amongst *E. coli* isolates from clinical samples.

Table 1 Source wise isolation of haemolytic *Escherichia coli* (*E.coli*)

S. No.	Source	Total samples	Total number of isolates	Number of *E.coli*	Number of haemolytic *E.coli*
1	Urine	12,941	2,764	1,295	181*
2	Blood	2,968	555	75	-
3	Pus	2,679	1,794	297	5
4	Throat swabs	826	172	21	-
5	Stool	469	346	163	7
6	Vaginal/cervical swabs	411	222	99	10
7	Sputum	294	90	20	-
8	CSF	188	4	2	-
9	Aural swabs	56	47	2	-
10	Nasal swabs	54	20	2	-
11	Others	2,121	195	25	2
	TOTAL	23,007	6,209	2,001	205

* x^2=46.62, p=<.001

Table 2 Age and sex wise distribution of haemolytic strains of *E.coli* (n=205)

S. No.	Source	Samples	Sex		Age in years							
			Male	Female	Up to 10 years		11-20 yrs.		21-40yrs.*		>40yrs.	
					Male	Female	Male	Female	Male	Female	Male	Female
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
1.	Urine	181 (88.29)	63 (34.80)	118 (65.19)	7 (11.11)	3 (2.54)	6 (9.52)	8 (6.77)	21 (33.33)	77 (65.25)	29 (46.03)	30 (25.42)
2.	Vaginal /Cervical Swab	10 (4.87)	-	10	-	-	-	-	-	8 (80.0)	-	2 (20.0)
			-		-	-	-	-	-		-	
3.	Stool	7 (3.41)	5 (71.42)	2 (28.57)	3 (60.0)	-	1 (20.0)	2 (100.0)	-	-	1 (20.0)	-
4.	Pus and Others	7 (3.41)	5 (71.42)	2 (28.57)	1 (20.0)	-	1 (20.0)	1 (50.0)	-	-	3 (60.0)	1(50.0)
	Total	**205**	**73 (35.60)**	**132 (64.39)**	**11 (15.06)**	**3 (2.27)**	**8 (10.95)**	**11 (8.33)**	**21 (28.76)**	**85 (64.39)**	**33 (45.20)**	**33 (25.0)**

N = number
(%) = Percentage
*x^2=<3.86, p=.05

Of 181 haemolytic *E. coli* strains from urine, 118 (65.19%) were from female patients and 63 (34.80%) were from male patients. Among stool, pus and other specimens 71.42% (5) were from male and 28.57% (2) were from female patients (Table 2). From urine and vaginal / cervical swab specimens, 65.25% and 80% of haemolytic *E. coli* strains were obtained from females in the age group of 21-40 years whereas among stool samples, 60% (3) strains were found to be haemolytic under 10 years of the age from male patients (Table 2).

Out of total 2,001 *E. coli* isolates, 10.24% were haemolytic, whereas Martinez *et al* (1999) reported 27.3% to be haemolytic amongst 207 isolates of *E. coli.* The incidence of haemolytic *E. coli* from stool and extra intestinal sources was found to be 4.29% and 10.77% respectively whereas others reported an incidence of haemolytic *E. coli* up to 60% from extra intestinal sources and 7% from faeces (Cavalieri *et al,* 1948).

In the present study, urine is the most frequently processed sample for culture and sensitivity. Of 1295 urinary *E. coli*

isolates, 181 (13.97%) were haemolytic which is found to be statistically significant (p< .001). The incidence reported by other workers varies from 29% to 80% (Banerjee, 1963; Bhalla qnd Aggarwal, 1989). But it appeared to approximate (11.11%) with the study done by Fule *et al* (1990). Kaur *et al* (1991) found 21.8% haemolytic strains from 152 urinary isolates. Vaisanen-Rhen *et al* (1984) found 60%, 27% and 17% strains that produced haemolysin in pyelonephritis, cystitis and asymptomatic bacteriuria cases respectively. Whereas Johnson (1997) found 49%, 40%, and 20% haemolytic *E. coli* in these conditions respectively.

As for as sex ratio is concerned, in the present study, 35.61% and 64.39% strains were haemolytic from male and female patients respectively (Table 2). Other workers observed no appreciable differences in male and female ratio (Geachie, 1966; Kumar and Khan, 1968). In the present study, though the incidence of haemolytic *E.coli* in females is high as compared to males but is statistically insignificant (p=.50). However, the highest incidence (51.7%) of these strains was in a age group of 21 to 40 years which is also statistically significant (p=.05). Out of these, 80.18% were from female patients. This indicates the incidence of haemolytic *E.coli* is more in the age group who are sexually more active and thereby more prone to urinary tract and genital tract infections.

The antibiogram pattern of the haemolytic *E.coli* isolated in the present study shows multiple drug resistance patterns. Antibiotic sensitivity testing revealed 53.75% strains to be multidrug resistant. Only 6.83% strains were sensitive to all the eight agents tested. About 13% strains were resistant to single drug and 23.43% strains were resistant to two drugs. On the whole, drugs that had shown good *in vitro* efficacy were cefoperazone (90.24%), netilmicin (83.90%) ciprofloxacin (74.14%), gentamicin (72.19%) and norfloxacin (71.70%).

In the present study, the drugs to which haemolytic *E.coli* had shown resistance were ampicillin (84.39%), co-trimoxazole (76.09%) and nalidixic acid (53.65%). It has been reported that as high as 52% of normal domiciliary population carry drug resistant *E.coli* in their bowel due to the indiscriminate use of antibiotics / chemotherapeutic agents, and not only due to R-plasmids (Dutta, 1969; Moorhouse, 1969). The resistance could be possibly due to the indiscriminate use of these drugs which play a major role in determining the resistance pattern of *E.coli* in bowel of patients and hence of urinary pathogens.

CONCLUSION

This study shows higher incidence of haemolytic *E.coli* from urine samples, particularly from females of sexually active age. At the same time these strains show considerable degree of multiple drug resistance. This organism being endowed with extra virulence tools, so there is need to have a greater awareness to this effect and such cases be treated more energetically.

Declarations

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