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Colonization with Antibiotic-Resistant *E. coli* in Commensal Fecal Flora of Newborns

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A B S T R A C T

Bacterial resistance to antibiotics represents one of the most significant global health challenges of this century. Although the role of commensal bacteria in providing a reservoir of antibiotic resistance has been acknowledged for some time these bacteria have not been studied extensively. The purpose of the present paper is to prospectively determine the presence of Multi drug resistant *E. coli* in commensal fecal flora of Newborns who are not receiving any antibiotics. After receiving parental consent 30 neonates less than 1month of age of either sex, hospitalized in the pediatric unit of NKP Salve Institute of Medical Sciences, not recently exposed to antibiotics were enrolled along with 30 neonates less than 1month of age admitted in NICU on treatment with various antibiotics. *E. coli* isolates from Stool samples were identified by standard biochemical tests & Antibiotic susceptibility was tested by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The Antibiogram of both the groups was compared to determine whether antibiotic therapy results in increase in the percentage of drug resistant *E. coli* population in the commensal fecal flora. In the newborns, who were not given antibiotics, resistance to Ampicillin was 100%, followed by Co-trimoxazole 27(96.42%). Highest sensitivity was observed to Amikacin 24(85.71%) followed by Gentamicin 16(57.14%).60% isolates were resistant to more than 3 drugs. In Newborns who were on various antibiotics, 100% resistance was seen to Ampicillin and Ceftriaxone followed by Co-trimoxazole 27(96.42%). Highest sensitivity was observed with Chloramphenicol 13(46.42). 11 isolates (39.28%) were sensitive to Amikacin and Tetracycline each. 90% isolates were resistant to more than 3 drugs.

Introduction

Bacterial resistance to antibiotics represents one of the most significant global health challenges of this century. Infections with multiple antibiotic-resistant bacteria have been increasing at an alarming rate owing to the widespread dissemination of antibiotic-resistance determinants (Collignon, 2002; O'Brien, 2002; Alekshun *et al*., 2006; Marshall *et al*., 2009) Although the role of commensal bacteria in providing a reservoir of antibiotic resistance has been acknowledged for some time (Alekshun *et al*., 2006; Marshall *et al*., 2009; Hawkey, 1986), these bacteria have not been studied extensively. *Escherichia coli* constitutes only a minor fraction of the bacterial flora found in the human gastrointestinal tract but is none the less an important reservoir. Increasing, incidence of high antibiotic resistance in commensal *E. coli* from healthy children and

adults from many countries has been documented by many recent studies (Jannine *et al*., 2010).

Hospital-associated Gram-negative bacterial colonization is acquired by newborns admitted to the intensive care unit throughout their NICU stay. It is most probably transmitted from the environmental flora via the hands of caregivers. Colonization specifically by antibiotic-resistant bacilli follows a similar pattern, suggesting that such bacteria make up part of the modern NICU ecology and are acquired by infants similar to susceptible microorganisms. Although some clinically-undetected cross-transmission of resistant bacilli occurs during non-outbreak periods, most colonizing antibiotic-resistant bacilli are unique to each infant (Toltzis, 2003).

Outbreaks with multidrug resistant bacteria usually result in treatment failure and also associated with increased mortality and higher costs. Adequate infection control practice, to limit horizontal spread of the bacteria and an early empirical antibiotic treatment effective against the causative bacteria is largely decisive for the outcome of neonatal septicemia (Toltzis, 2003).

The purpose of the present paper is to prospectively determine the presence of Multi drug resistant *E. coli* in commensal fecal flora of neonates who are exposed to antibiotics versus those who are not exposed to antibiotics and also to determine the extent of problem in our region.

Materials and Methods

After clearance from Institutional ethics Committee, a cross sectional study was done. All neonates admitted in the NICU and SICU of NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital, Digdoh from June 2014 to September 2014 (3 months) was enrolled in the study (As the study was part of ICMR-STS project)

Total 60 neonates were enrolled in the study, after receiving parental consent

Inclusion criteria

All neonates less than 1 month of age admitted in the hospital.

Exclusion criteria

Baby born to immune compromised mother.

The neonates were divided into 2 groups

Group A: 30 neonates less than 1month of age of either sex, hospitalized in the pediatric unit of NKP Salve Institute of Medical Sciences, not recently exposed to antibiotics were enrolled along

Group B: 30 neonates less than 1month of age admitted in NICU on treatment with various antibiotics.

Methods

Medical history (prior hospitalization, prior invasive devices used, Mode of delivery), antibiotic therapies, diagnosis on admission and discharge was recorded in case record form. Patient Stool samples were collected and immediately transported to microbiology laboratory.

Laboratory methods

Stool samples were immediately plated onto Mac-conkeys agar. The plates were kept in incubation at 37°C for 24- to 48 hours. The *E. coli* isolates were identified by Gram staining and by standard biochemical tests. *E. coli* isolate from each stool sample was subjected to antimicrobial susceptibility testing using Kirby-Bauer disc diffusion method. CLSI interpretative criteria for susceptibility and

resistance were used, ATCC *E. coli* 25922 was simultaneously tested as control with each batch of antimicrobial susceptibility testing performed (Clinical Laboratory Standards Institute, 2011). After verifying the results of the standard strain, test sample results were interpreted. Zone diameters were read using antibiotic zone scale (Himedia, Mumbai, India).

Following antibiotics discs were tested (Hi-Media, Mumbai), Ampicillin (10mcg), Co-trimoxazole (25 mcg), Gentamicin (30 mcg), Amikacin (30 mcg), Tetracycline (30 mcg), Chloramphenicol (30 mcg), Ceftriaxone (30 mcg), Ciprofloxacin (5 mcg).

Data analysis

The above collected data was analyzed by proportion rate method.

Results and Discussion

The number of *E. coli* isolates obtained from the neonates who were not exposed to antibiotics was 28 (Group A) and similar number of *E. coli* isolates were obtained from the neonates who were admitted to NICU and were on various antibiotics (Group B). Two stool samples did not show growth of *E. coli* from both the groups.

In neonates belonging to group A, all isolated *E. coli* were resistant to Ampicillin (100%), followed by co-trimoxazole 27(96.42%). Most of the isolates were sensitive to d to Amikacin 24(85.71%) followed by Gentamicin 16(57.14%). Multi drug resistance (more than 3 drugs) was seen in sixty percent of isolates (Table 1).

In neonates belonging to group B, all isolates were resistant to *E. coli* and Ceftriaxone, followed by co-trimoxazole 27 (96.42%). Highest sensitivity was observed with Chloramphenicol 13 (46.42%).

Eleven isolates (39.28%) were sensitive to Amikacin and Tetracycline each. Multi drug resistance (more than 3 drugs) was seen in Ninety percent isolates (Table 2).

Almost all the neonates admitted to a NICU belonging to group B were on various antibiotics (Table 3) shows the antibiotics administered to the neonates during their admission. The initial empirical antibiotics administered were Vancomycin and Ceftriaxone. The antibiotics were changed after culture and sensitivity reports.

Fifty-seven neonates in the present study were delivered by c-section and three neonates were by normal delivery. All the mothers who went through C-section were given Injection Ceftriaxone and Injection Metronidazole as prophylactic antibiotics one hour before operation (Table 4).

Irrational use of antibiotics as prophylaxis or for empirical use results in exposure of microorganism to antimicrobial agents. Such use of an antimicrobial agent selects for overgrowth of a bacterial strain that has a gene expressing resistance to the agent. The levels of resistance to antimicrobial agents at any time and place may therefore reflect in part the total number of bacteria in the world exposed to antimicrobials up until then. There is widespread indirect dissemination of antimicrobial resistance from varied sources, this is evident from studies on microbial population biology, microbial genetics, and clinical and epidemiological observations (Karine *et al*., 2004).

In the present study 100% resistance was observed to Ampicillin and 96% resistance was observed to Co-trimoxazole in Group I.

Most of the isolates were sensitive to Amikacin (Table 1).

Dyar *et al.*, conducted a study in rural community of Vietnam, high degree of resistance was found in the *E. coli* isolated from feces to Ampicillin (65%), Tetracycline (74%) and Co-trimoxazole (68%). Resistance to Chloramphenicol and Nalidixic acid was significantly low (10) In the study by Shanahan *et al.*, 88.6% *E. coli* were resistant to Ampicillin, followed by Trimethoprim (74.2%). Chloramphenicol resistance was found to be lower at 52.6% while the carriage of Gentamicin and Nalidixic acid resistance were lower still at 7.5°% and 10.2 % respectively.(11) In the rural community of French Guyana, Karine grenet *et al*., (2004) observed that *E. coli* isolates were mostly resistant to Amikacin (55%) and Tetracycline (63%) followed by Streptomycin (45%). Twenty nine and twenty four percent isolates were resistant to Co-trimoxzole and Cholramphenicol respectively. All isolates were sensitive to third generation Ceftriaxone and Nalidixic acid.

In a study by Garcia *et al.*, (2011), total 220 strains were recovered from the fecal specimens (61.8% from acute diarrhoea and 38.2% from without diarrhoea). The isolates showed high resistance rates against ampicillin, tetracycline, and sulfamethoxazole-trimethoprim.

In another study, by Oluyege *et al.*, (2015) The *E. coli* isolates exhibited 90.2% and 88.2% resistance to amoxicillin and tetracycline respectively while over 75% were resistant to cotrimoxazole and augmentin. Ninety percent (90%) of *E. coli* isolated had multiple resistance to three or more antibiotics. There was carriage of multiple resistant commensal *E. coli* irrespective of exclusive or inclusive mode of feeding, or previous history of use of antibiotics or other parenteral drugs. Carriage of multiply resistant commensal *E. coli* was present even in infants with no history of use of any drug.

In a meta analysis by Bryce *et al*., (2016), 34 research paper of carriage of antibiotic resistant fecal *E. coli* were analysed along with comparison of economic status on basis of OECD status. The study showed high rates of faecal *E. coli* resistance in asymptomatic children, the resistance rate was higher in non-OECD countries and association was found between primary care prescribed antibiotics and resistance lasting for up to 3 months post-prescribing. Sheidman *et al*., (2009) demonstrated that 32% of *E. coli* was resistant to more than three antibiotics.

In a study by Shakya *et al*., (2013) for antibiotic resistance patterns in *E. coli* isolates from stool samples of children aged 3-14 years from Ujjain, antibiotic resistance patterns in *E. coli* isolates, overall, 72% of isolates were resistant to at least one antibiotic and 33% were multi-drug resistant. Proportions of isolates resistant to various antibiotics were, nalidixic acid, (45%), tetracycline (37%), ampicillin (37%), sulfamethoxazole/trimethoprim (29%) and amoxicillin/clavulanic acid (29%). High rates of cross-resistance were seen for 15 (83%) of the antibiotics studied. No isolates were resistant to imipenem.

Thus Studies done by various authors have demonstrated higher resistance to Ampicillin and Co-trimoxazole in the commensal fecal flora. The sensitivity towards Aminoglycosides and Fluroquinolone was varying from 80% to 50 %.

The findings in our study for Group 1 are similar to most of the other studies which have studied *E. coli* isolates in fecal flora from community and shows that most of the *E. coli* isolates in community show high degree of resistance to Ampicillin and Co-

trimoxazole. The studies also show that multidrug resistance (resistance to more than 3 drugs) is common phenomenon in various parts of the world.

In the present study in Group II, most of the isolates were resistant to Ampicillin and Ceftriaxone. The resistance to Amikacin and Ciprofloxacin was also significantly increased in this group as compared to Group I (Table 2) ($p < 0.5$). Study conducted in Indonesia at Fatimawati hospital by Maksum Radji, Siti Fauziah, Nurgani Aribinuko for Antibiotic sensitivity pattern of bacterial pathogens in the intensive care unit showed resistance pattern as Ceftriaxone (46%), Amikacin (15.4%), Gentamycin (38%), Ciprofloxacin (46.2%). In a study performed by Todisoa Andriatahina *et al.*, (2010) fecal carriage of Multi drug resistant *E. coli* was detected in 21.2% of 244 infants on admission and 57.1% of 154 on discharge, after more than 48 hours of hospitalization ($p < 0.001$). *E. coli* which was ESBL-positive showed high degree of resistance to Trimethoprim-Sulfamethoxazole (91.3%), Gentamicin (76.1%), Ciprofloxacin (50.0%), but not to Amikacin and Imipenem. The increased prevalence of carriage during hospitalization was related to standard antimicrobial therapy.

Table.1 Antibiogram of *E. coli* isolates obtained from the neonates who were not exposed to antibiotics

ANTIBIOTICS	RESISTANT N=28
AMPICILIN	28 (100%)
CO-TRIMOXAZOLE	27 (96.42%)
CEFTRIAXONE	12 (42.85%)
GENTAMICIN	12 (42.85%)
AMIKACIN	4 (14.28%)
CIPROFLOXACIN	16 (57.14%)
TETRACYCLINE	21 (75%)
CHLORAMPHENICOL	16 (57.14%)
TOTAL	28(100%)

Table.2 Antibiogram of *E. coli* isolates obtained from the neonates admitted to NICU and were on various antibiotics

ANTIBIOTICS	RESISTANT (N=28)
AMPICILIN	28(100%)
CO-TRIMOXAZOLE	22(78.57%)
CEFTRIAXONE	28(100%)
GENATAMICIN	20(71.42%)
AMIKACIN	17(60.71%)
CIPROFLOXACIN	21(75.00%)
TETRACYCLINE	17(60.71%)
CHLORAMPHENICOL	15 (53.57%)
TOTAL	**28 (100%)**

Table.3 Antibiotics administered to neonates admitted to NICU

Name of Antibiotics	Percent of newborns
Vancomycin	100%
Ceftriaxone	50%
Amikacin	70%
Meropenem	100%

Table.4 Antibiotic administered to mother during cesarean section as prophylactic antibiotics

Name of antibiotics	No (%)
Ceftriaxone	100%
Metronidazole	100%

Dethlefsen *et al*., (2008) in a study demonstrated that administration of ciprofloxacin, a commonly used antibiotic with little activity against obligate anaerobic bacteria, altered the abundance of roughly one third of intestinal bacteria in three healthy volunteers, with a significant loss of microbial diversity during treatment.

Most of the studies done in the hospitalized population have shown that the *E. coli* in the commensal flora of the hospitalized patients acquires resistance due to selective pressure of the antibiotics. The antibiogram depends on the antibiotics routinely used in the hospital.

In our hospital the antibiotics used for prophylaxis were Cephalosporin (Ceftriaxone) and Glycopeptides (Vanco-mycin). We have tested the isolates for their resistance against cephalosporin and our data shows that the number of isolates resistant to Ceftriaxone is significantly more in the Group II than in the Group I ($p < 0.5$)

Also the use of Ceftriaxone as prophylactic antibiotics in the mothers may have influenced the resistance pattern to the *E. coli* in commensal fecal flora (Table 4)

The isolates in group 2 were ninety percent multidrug resistant (Table 2). Fecal *E. coli* is regarded as a useful indicator of the spread of acquired antibiotic resistance genes in the community, Commensal Escherichia coli can act as reservoirs of resistance genes in the human gut. These resistant genes might be rapidly transferred to other commensal or pathogenic organisms. Hence proper antibiotic policy and restricted use of higher antibiotics is need of hour.

In conclusion, our study demonstrated the high prevalence of resistance to individual antibiotics in commensal *E. coli* isolated from both groups of newborns admitted in neonatal intensive care unit. The increased empirical use of antibiotics has caused multidrug resistant *E. coli* to emerge even in the commensal flora of community hence it is imperative to use antibiotics judiciously. As the study period was limited, number of isolates studied was less hence study with larger population is required to completely assess the impact of antibiotics on commensal fecal flora.

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