Volume-4, Issue-5, May-2015 • ISSN No 2277 - 8160

Research Paper Microbiology

Virulence Factors, Antimicrobial Resistance and Beta-Lactamase Production Among Extra-Intestinal E.coliisolates from Various Clinical Samples

Priya R MSc clinical Microbiology

Kalaivani R Assistant Prof, Department of clinical Microbiology, MGMCRI, Pondicherry

Seetha K.S Professor&HOD , Department of clinical Microbiology, MGMCRI,Pondicherry

ABSTRACT

INTRODUCTION:

E.coli is one of the commonly isolated aerobic pathogen in all clinical Microbiological laboratories. Though it is a normal inhabitant of human beings, by acquiring antimicrobial resistance it causes a very wide range of morbidity and mortality. Multidrug resistance is of great burden to the healthcare facility providers. Proper antibiotic sensitivity testing, identifying its various resistance and virulence mechanisms and judicious use of antimicrobial agents will reduce the burden.

MATERIALS AND METHODS:

A total of non-repetitive 100 E.coli isolates from various extra intestinal E.coli infections were included in this study for a period of two months from a tertiary care hospital. Following isolation their antimicrobial resistance pattern and various mechanisms of resistance like virulence factors and beta lactamase production were done according to standard guidelines with ATCC E.coli 25922 as quality control strain.

RESULTS:

From the total of 100 E.coli isolates, the majority (61%) were from urine samples followed by others. Seventy two percentages of our isolates were positive for haemagglutination virulence factor. Gelatinase production was seen among 52% of E.coli. Highest percentage of resistance was observed towards ampicillin (89%) and ciprofloxacin 79% followed by others. Least percentage of resistance was noticed towards Imipenem, colistin and polymyxin B. ESBL and AmpC beta lactamase production was seen among 41% and 9% respectively.

CONCLUSION:

As antimicrobial resistance is an great burden to withstand its morbidity and mortality, active surveillance programmers and proper documentation will plot a way in the future to overcome its difficulty.

KEYWORDS : Virulence factor, ESBL, AmpC.

INTRODUCTION:

Escherichia coli is a ubiquitous and diverse organism and it is one among the commonly isolated nosocomial and community-associated pathogen.[1,2] Although it is a normal intestinal commensal and inhabitant of human beings and warm-blooded animals, by rendering various virulence factors and resistance mechanisms it causes pathogenesis, which includes, UTI, enteric diseases, sepsis, meningitis and wound infections.[3] Major virulence factors involved in the pathogenesis are cell surface-modifying factors, invasions, toxins, secretion systems, hemolysin production, siderophore production, aerobactin, serum resistance factors, cytotoxic necrotizing factor(CNF), capsule production and uropathogenic specific C protein, etc., The property of adherence to the uroepithelium is mediated by Fimbriae in case of uropathogenic*E.coli*.[1,4,5,6,7]. Virulence genes helps in the bacterial colonization, especially in the urinary epithelium (UPEC) and causes acute to severe disease manifestation and antimicrobial resistance.[8]

Virulent strains develops high rate of antimicrobial resistance leading to a therapeutic challenge.[8] Antimicrobial resistance may be plasmid as well as chromosomal mediated.[9] Increase in antimicrobial resistance is a major concern worldwide. Multi-drug resistant isolates were found to be of great challenge to the health-care facility providers. Among the MDR *E.coli*isolates, ESBL and AmpC beta-lactamase production were found to be the prevalent resistance mechanisms.[10,11] Thus this study was aimed to detect various virulence factors and predominant phenotypic resistance mechanisms like ESBL and AmpC production among various extra-intestinal *E.coli* isolates.

MATERIALS AND METHODS:

BACTERIAL STRAINS: A total of consecutive and non-repetitive 100 extra-intestinal *E.coli* isolates isolated from various extra-intestinal clinical samples for a period of two months from Mahatma Gandhi medical college and research institute, SBV University were included in the study.

SPECIMEN COLLECTION: All the Clinical specimens which includes pus, exudates, clean catch midstream urine, sputum, endotracheal aspirates, blood, cerebrospinal fluid, aspirates were collected in a sterile container with proper aseptic measures.[12] Repeated isolation of E.coli from the same patients, intestinal samples and Foley's catheter tips were excluded from this study. The samples were processed immediately as per standard operative procedures and guidelines.[13]

SAMPLE PROCESSING: The samples were processed and identified based on Gram's staining, culture characteristics and biochemical reactions. And the antibiotic susceptibility testing was carried out by Kirby Bauer disc diffusion method (CLSI) with ATCC *E.coli 25922* isolates as quality control strain for all the procedures.

METHODOLOGY:

With all the 100 extra-intestinal *E.coli*isolates, the following methods were carried out.

DETECTION OF VIRULENCE FACTORS:

HEMOLYSIN PRODUCTION:

Isolated *E.coli* was inoculated on to 5% sheep blood agar plate and incubated at 37°C for 24 hrs. A zone of complete lysis of erythrocyte around the colony and clearing of medium occurs if hemolysin is produced by *E.coli* isolates.[14]

GELATINASE TEST:

Gelatin agar was used to detect the gelatinase production by *E.coli*. In an agar plate the test organism was inoculated and incubated at 37°C for 24 hours and then the plates was flooded with mercuric chloride solution. Formation of opacity in the medium and zone of clearance developing around the colonies were considered as positive.[13]

HAEMAGGLUTINATION:

Haemagglutination test was detected by clumping of erythrocytes by fimbriae of *E.coli* in the presence of Dmannose. *E.coli* strains were inoculated on to 1% nutrient broth and incubated at 37˚ °C for 48hrs for full fimbriation. Human blood group `O´ was taken and it was washed thrice in normal saline and made up to 3% suspension. It was used immediately with in the week by storing at 35˚ ˙ °C. The slide Hae-

magglutination test was carried out on a multiple concavity slide, by adding one drop of RBC suspension to a drop of test broth culture and the slide was rocked to and fro for 5mins. Presence of clumping was taken as positive for Haemagglutination. Mannose sensitive haemaggluination was detected by the absence of agglutination in a parallel set of test in which a drop of 2% w/v Dmannose was added to the suspension. Mannose resistant haemaggluination was detected by the presence of haemaggluination of 3% `O´ RBC in the presence of 2% mannose.[15]

ANTIBIOTIC SUSCEPTIBILITY TESTING:

The antibiotic susceptibility testing was done using Kirby Bauer disc diffusion method on Mueller Hinton agar. The turbidity of the inoculum was matched 0.5 McFarland's Turbidity standard and lawn cultures were prepared. For quality control, *E. coli ATCC 25922* strain was used. Once the surface was dried, the antibiotic disks such as Ampicillin, Gentamicin, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Amikacin, Piperacillin/Tazobactam, Cefotaxime/ Ceftriaxone, Imipenem, Polymixin B and Colistinwere placed over it. In case of urinary isolates Nalidixic acid, Norfloxacin, Nitrofurantoinwere also included. After 18 hours of incubation at 37°C, the inner diameter of the zone of inhibition was measured by using a millimeter scale. The zone size around each antimicrobial disk was interpreted as sensitive, intermediate or resistant according to the CLSI guidelines. [16]

PHENOTYPIC RESISTANCE MECHANISMS:

Phenotypic resistance mechanisms like, ESBL production by Double disc synergy test and AmpC detection by AmpC disc method were also carried out for all these 100 extra intestinal E.coli isolates.[17]

RESULT:

A Total of 100 *E.coli* isolates were obtained from the patients with extra intestinal infections. Out of these, 61 strains were from urine, 23 from exudate, 10 from respiratory and 6 from blood samples respectively. (Fig.1)

Among these 100 *E.coli* isolates according to age wise distribution of the patients, the majority of *E.coli* were isolated between the age 26 and 45 years (32%), 27% and 23% were from 46 to 65 years and 18 to 25 years respectively, followed by others. (table 1) According to sex distribution of *E.coli* isolated from various clinical samples showed female preponderance of 67% and 33% were from male patients.

Among various virulence factors studied, Haemagglutination was observed in 72 isolates, in which 30% were mannose sensitive haemagglutination (MSHA) (type1 fimbriae) and 42% were mannose resistant haemagglutination (MRHA) (p-fimbriae) type. Gelatinase production was noticed in 52 isolates and none of these 100 isolates produced hemolysin production. (Fig 2)

Antimicrobial resistance pattern against various antibiotics showed, 89% resistance towards Ampicillin, 79% towards Ciprofloxacin, Gentamicin 68%, Trimethoprime/Sulfamethoxazole 75%, Amikacin 48%, Piperacillin/Tazobactam 53%, Cefotaxime/ Ceftriaxone 78%,Imipenem 3%, Polymixin B 2% and 3% towards Colistin. Seventy six percentage of these*E.coli* were Multidrug resistant strains. (Fig 3) Among 61 urine isolates, Nalidixic acid, Norfloxacin, Nitrofurantoin showed 85%, 79% and 41% resistance respectively.

Among Gram negative bacilli, ESBL and AmpC beta-lactamases productions were found to be predominant mechanisms of resistance. Out of our 100 *E.coli* isolates, 34 urine isolates, 6 exudate and 1 respiratory isolates were found to be ESBL producers. AmpC beta-lactamase production was observed in seven urine, one exudate and one sputumisolates. None of the total six blood isolates showed either ESBL or AmpC production. (Table 2)

DISCUSSION:

E.coli is a known potent nosocomial and community acquired pathogen which possesses virulence factors that allow it to invade, colonize and induce pathogenesis in various anatomical sites. Among the wide variety of diseases, extra-intestinal infections, urinary tract infection, soft tissue infection and bacteremia are of great health burden. (9,10,11) Among our 100 E.coli isolates, UTI was the predominant source (61%) of isolates, which was followed by 23% of exudate samples which were very similar to other studies.(18,3,4)

In this present study, among the total 100 extra intestinal *E.coli* isolates, isolates from females patients were found to be predominant source (67%) when compared to male patients (33%). And the majority were between the age group of 26-45years of age. Study by Shruthi N et al between 2009 & 2010 and Ibrahim ME et al (2011) also reported female predominance which was very much comparable with our findings. [19,5] According to Shruthi N et al., the majority (39%) of *E.coli* were isolated from patients in the age group of 21-30 years followed by 61-80years of age, with a preponderance of female. [19] Female predominance may be because of the anatomical differences like short urethra, shorter distance between the anus and ureteral opening and moist perineum.[20]

Though majority of our *E.coli* isolates were from urinary samples, we have not documented even a single hemolytic *E.coli* isolates producing any of the three types of hemolysin such as α, β and γhemolysin from all of our clinical samples. The cytolytic protein toxin secreted by most hemolytic *E.coli* as hemolysin, may contribute to tissue injury to the survival of organism in renal parenchyma and entry in to the bloodstream.[21,22] Desai S et al and Minshew et al have reported 54% and 49% α hemolysin production from their isolates respectively.[23,24] A study by SaiSwaroop et al have reported presence of 40% hemolysin production, all these findings were very much contrast to our results.[6]

The Fimbriae mediates haemagglutination and adherence property. [14]Haemagglutination inhibited in the presence of D-mannose was labeled as mannose sensitive haemagglutination (MSHA) indicating type 1 fimbriae and if agglutination occurred even in the presence of D-mannose, it was represented as mannose resistant haemagglutination (MRHA) due to p fimbriae, and they were considered as UPEC. [15,23,25] Among our *E.coli*collections, 30% of them showed mannose sensitive type haemagglutination (MSHA) and 42% showed mannose resistant type haemagglutination (MRHA). Desai S et al (2013) reported 30% of mannose sensitive haemagglutination (type1 fimbriae) and 36% of mannose resistant haemagglutination(p-fimbriae) with their *E.coli* isolates.[23] This was very much similar to our findings.

Yet in another study by Green PC et al MRHA was demonstrated in 56% of *E.coli* isolates.[25] Johnson et al reported 52% MRHA E.coli with group A positive erythrocytes.[20] Their findings were very much close to our results. In the present study, Gelatinase production was observed in 52% of E.coli isolates. Shruthi N et al reported 19.4% and Johnson et al also reported only 7% gelatinase production, which were not correlating to our findings and it was not comparable to others.[27,20]

Many factors influence the antimicrobial resistance among the bacterial pathogens. Of which, injudicious and inadequate use of antimicrobial agents plays a pivotal role in the incidence of Multi drug resistance.[8,11]Seventy six percentage of these *E.coli* in the present study were Multidrug resistant strains (76). (Fig 3) Which is of great burden to the clinicians. The highest percentage of antimicrobial resistance was towards Ampicillin (89%) followed by this, Ciprofloxacin (79%), Gentamicin (68%), Trimethoprim/Sulfamethoxazole (75%), Amikacin (48%), Piperacillin/Tazobactam (53%). Least percentage of resistance was documented towards Imipenem and Colistin (3%) finally 2% against Polymixin B. Out of 61 urine isolates, towards Nalidixic acid, Norfloxacin, Nitrofurantoin they showed 85%, 79% and 41% resistance respectively. Towards 3rd generation cephalosporin's either Cefotaxime/ Ceftriaxone 78% resistance was noticed. ESBL confirmatory test results showed 41% ESBL production. AmpC production was seen among 9% of our *E.coli* isolates. Out of all clinical samples, ESBL production was maximum towards urinary isolates. Similarly, the major percentage of AmpC producers were from respiratory samples.

Ibrahim ME et al reported 92.2% of MDR *E.coli* isolates. Similarly prevalence of MDR *E.coli* from various countries like Sudan (58%), Egypt (74.6%), Ethiopia (83.9%), Nigeria (74%) and from Saudi Arabia 74% have also got documented.[5] Whoever all this data's are very much comparable to our MDR *E.coli* prevalence. AsimaBanu et al with 238 urinary *E.coli* isolates reported the maximum number of resistance towards ampicillin 94.1% followed by 67.6% resistance against cotrimoxazole, 47.4% gentamicin, 32% Cefotaxime and the least per-

centage of resistance towards ciprofloxacin (19%), which was very similar to the present study.[5] In contrast to our study SaiSwaroop et al have reported 93% resistance towards Imipenem and towards Amikacin 91% resistance.[6] Desai S et al reported 30% of ESBL production among their *E.coli* isolates which was closely similar to our findings.[23]

CONCLUSION:

As *E.coli* is one of the important pathogen to cause a wide range of illness, it is very significant to have an active surveillance measures to know all its emerging antimicrobial resistance mechanisms. So that appropriate drug management can be planned and executed. To start with any empirical antibiotics, it is necessary to know all the mechanisms, virulence properties and antimicrobial resistance pattern by which this pathogen can withstand the pressure. The goal to prevent the dissemination of drug resistant pathogen, can be achieved mainly by promoting various research activities and by putting an end to indiscriminate use of antibiotics. Thus proper pathogen identification, its characterization, providing good quality reports, judicious use of antibiotics and by implementing appropriate antibiotic policy, will surely help to strengthen and to take necessary steps to prevent the spread of diseases and would render necessary treatment options for the sufferers against *E.coli* and other similar potent pathogens.

Tables & figures
Legends:
Table 1:Age distribution
Table 1:

Age distribution	Number of Extra intestinal *E.coli* isolates
0-11 months	03
12 months – 17 years	09
18 – 25 years	23
26 – 45 years	32
46 – 65 years	27
Above 65 years	06

Table 2:Distribution of extra-intestinal E.coli isolates and percentage of Beta- lactamase production

Table 2: Distribution of extra-intestinal *E.coli* isolates and percentage of Beta-lactamase production			
Isolated*E. coli*isolates	TOTAL (n=100)	ESBL production(n=41)	AmpC production(n=9)
Urine samples	71	47.88%(34)	9.85%(7)
Exudate samples	23	26%(6)	4.3%(1)
Respiratory samples	10	10%(1)	10%(1)
Blood samples	6	0	0

Fig 1:Sample wise distribution of extra intestinal E.coli

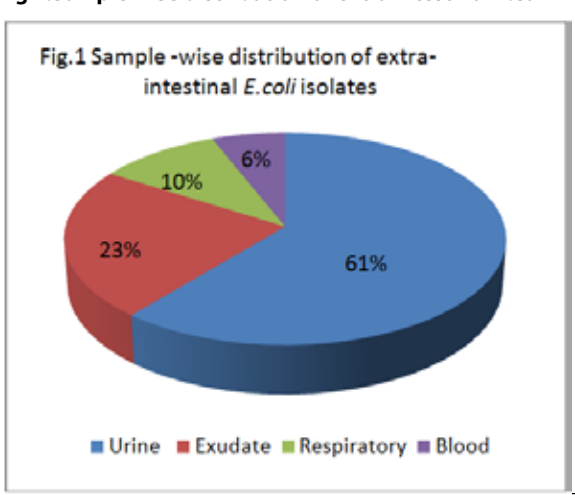
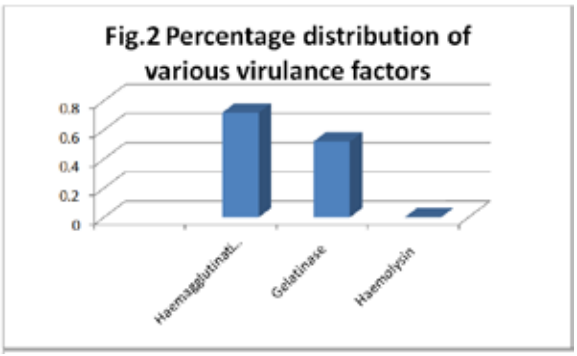


Fig 2:Percentage distribution of various virulence factors



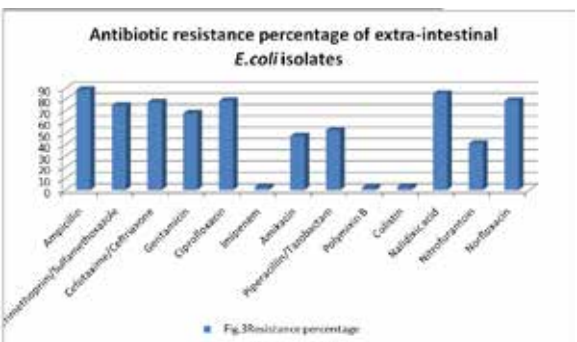


Fig 3:Antibiotic resistance pattern of extra intestinal E.coli isolates

REFERENCES

1. Ivan Holko, Tatiana Bisova, ZuzanaHolkova, Vladimir Kmet. Viruence markers of E.coli strains isolated from traditional cheeses made from unpasteurized sheep milk in Slovakia. Food control 2006;17:393-396. | 2. Barati S, Boniadian M, Habibian R, Jostejou T. Antibiotic resistance of enterotoxigenic and entroaggrigativeE.coli isolated from gastroenteritis cases. Asian journal of biomedical and pharmaceutical sciences 2012;2(14):54-58. | 3. AsimaBanu, Jyoti S Kabbin, MriduAnand. Extraintestinal infections due to E.coli: An | emerging issue. Journal of Clinical and Diagnostic research 2011 june;5(3):486-490. | 4. Sasakawa C, Kacker J. Host-microbe interaction:bacteria.Curr Op Microbiol 2006;9:1-4. | 5. Ibrahim ME, Bilal NE, Hamid ME. Increased multi-drug resistant Escherichia coli from hospitals in Khartoum state, Sudan. African Health sciences 2012;12(3):368-375. | 6. SaiSwaroop P, HemaPrakashKumari P and SrinivasaRao U. Virulence-associated factors in Escherichia coi strains isolated from urinary tract infections. Int.J.Curr.Microbiol.App.Sci 2013;2(10):436-440. | 7. Silveira W, Benetti F, Lancelotti M, Ferrerira A, Solferini V, Brocchi M. Biological and genetic characteristrics o uropathogenicE.coli strains. Rev. Inst. Med. Trop. Sao Paulo 2001;43:33-310. | 8. Gulsun S, Oguzoglu N, Inan A, Ceran N. The virulence factors and antibiotic sensitivities of E.coi isolated from recurrent urinary tract infections. Saudi Med 2005;26:1755-1758. | 9. Johnson JR. The virulence factors in the E.coli which were isolated from urinary tract infections. ClinMicrobiol Rev. 1991:4(1):80-128. | 10. Christina FM van der Donk, Jeroen HB van de Bovenkamp, Els IGB De Brauwer, Patrick De Mol, Karl-Heinz fedhoff, et al. Antimicrobial resistance and spread of multidrug resistant E.coli isolates collected from nine Urology services in the Euregion Meuse-Rhine. PLOS ONE 2012, October;7(10):e47707(1-5). | 11. Deshmukh PM and Ukesh CS. Multidrug resistant pattern of E.coli isolated from urinary tract infected (UTI'S patients.Int. J of Life Sciences 2014;2(1):53-57. | 12. Thomson Jr, RB, Miler JM. Specimen transport and collection, processing: bacteriology. In Manual of Clinical Microbiology. 8th edition, edited by Murray PR, Baon EJ, Pfaler MA, Tenover FC and Yolen RH., Washington DC : American society for Microbiology 2003; 286-330. | 13. Farmer JJ 3rd. Enterobacteriacea: Introduction and identification. In: Manua of Clinical Microbiology. 8th edition, edited by Murray PR, Baon EJ, Pfaler MA, Tenover FC and Yolen RH., Washington DC : American society for Microbiology 2003; 636-653. | 14. Duguid JP, Clegg S, Wilson MI. The fimbrial and the non-fimbrialhaemagglutinations of E.coli. J Med Microbiology 1979;12:213-27. | 15. Johnson JR. The virulence factors in the E.coli which were isolated from urinary tract infections. ClinMicrobiol Rev. 1991:4(1):80-128. | 16. Cinical and laboratory standards institute. Performance standards for Antimicrobial disk susceptibility testing; Twenty-third informational supplement. CLSI Document M100- 2013 January;S23,vo.31(1). | 17. Kalaivani R, Shashikala P, Sheela Devi C, Prashanth K, Saranathan R. Phenotypic assays for detection of ESBL and MBL producers among the clinical isolates of multidrug resistant Pseudomonas aeruginosa from a tertiary care hospital. Int J Cur Res Rev 2013 Sep;05(17):28-35. | 18. Olowe OA, Okanlawon BM, Olowe RA and Olayemi AA. Antimicrobial resistant pattern of E.coli rom human clinical samples in Osogbo, South western Nigeria. African Journal of Microbiology Research 2008; 2:08-11. | 19. Shuruthi N, Ravikumar, Ravishkumar. Phenotypic study of virulence factors in Escherichia coi isolated from antenatal cases, catheterized patients and faecal flora. JCDR 2012 December;6(10):1699-1703. | 20. Johnson JR. The microbial virulence determinants and the pathogenesis of urinary tract infections. Infect Dis Clin N Am 2003;17:261-78. | 21. Cavalieri SJ, Bohach GA and Snyder IS. Escherichia coli alpha haemolysin: characteristics and probable role in pathogenicity. Micobiol. Rev. 48:326-343. | 22. Chen SL, Hung CS, Xu J, Reigstad CS, Magrini V, Sabo A. Identification of genes subject to positive selection in uropathogenic strains of E.coli: a comparative genomics approach. Proc.Natl.Acad.Sci. USA,2006;103:5977-5982. | 23. Desai S, Rajput A, Kagal A, Bharadwaj R. Virulence factors in uropathogenicE.coli causing urinary tract infections. Indian Journal of basic and applied Medical research 2013 September;8(2):886-896. | 24. Minshew BH, Jorgensen J, Swanstrun M, Grootes-Reuvecamp. Some characteristrics of Escherichia coli strains isolated from extraintestinal infections of humans. J Infect Dis 1978;137:648-654. | 25. Seigfried L, Kmetova M, Puzova H, Molokacova M, Filka J. The virulence associated factors in the Escherichia coli strains which were isolated from children with urinary tract infections. J Med Microbiology 1994;41:127-52. | 26. Green PC, Thomas VL. Haemagglutination of human type O erythrocytes. Hemolysin production | 27. Shuruthi N, Ravikumar, Ravishkumar. Phenotypic study of virulence factors in Escherichia coi isolated from antenatal cases, catheterized patients and faecal flora. JCDR 2012 December;6(10):1699-1703.